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FURTHER TESTS OF INSECTICIDES AGAINST BLACK FLIES (DIPTERA: SIMULIIDAE) AND A CONTROL PROCEDURE[1]

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INTRODUCTION

Preliminary investigations into the possibility of controlling black flies in the larval stage with some of the synthetic organic insecticides were made at Churchill, Manitoba, in June and July, 1947, and have already been reported (5). This work was continued at Churchill and in the vicinity of Whitehorse, Y.T., in 1948 and 1949, with special attention to the possibility of obtaining a satisfactory mortality of the eggs and pupae, and of arriving at a practicable procedure for routine control measures.

During this time, similar work on Alaskan species has been reported. Gjullin and his co-workers (3, 4) obtained control of the larvae of *Simulium venustum* Say, *S. vittatum* Zett., and *Eusimulium latipes* (Mgn.) with dosages of various DDT formulations from 4.5 to 10.5 p.p.m.-minutes, and reported that *Prosimulium hirtipes* Fries could be detached by a dosage of 1.8 p.p.m.-minutes, which closely approaches that found satisfactory against *S. venustum* at Churchill in 1947. The same workers obtained satisfactory results from aerial applications. Using methoxychlor and dichlorodiphenyl dichloroethane, Travis (11) has reported results comparable to those obtained with DDT; he also reported parathion more effective than other materials, but not sufficiently so to warrant its use. Travis considered DDT in fuel oil solution the most satisfactory formulation, applied for an unspecified time at 0.4 p.p.m., and recommended aerial spraying as a method of application. The species referred to in his paper are *S. venustum*, *S. vittatum*, and *Prosimulium fulvum* Coq. Australian workers (8) have reported methods for the laboratory rearing of black flies, and have shown interest in aerial applications of DDT. Work is planned on the control of *Simulium neavei* Roub. over a large area in East Africa, in connection with onchocerciasis (2). In Canada, *Simulium arcticum* Mall. has been controlled, for a proved distance of 17 miles and a possible distance of 90 miles downstream, by a single aerial application of DDT in fuel oil at 4.68 p.p.m.-minutes, but 2.38 p.p.m.-minutes failed to give control (1).

[1] The results herein reported are from a research project which forms part of a program of studies on the biology and control of biting flies in northern Canada being carried out by the Division of Entomology, Science Service, Canada Department of Agriculture, on behalf of the Defence Research Board, and with the co-operation of that organization and other agencies.

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MATERIALS TESTED

Tests were made against one or more of the aquatic stages of black flies, and against other ecologically related organisms in the same environment, with each of the following materials:—

DDT (Technical Grade):

10 per cent W/V solution in fuel oil plus Velsicol A.R. 50 as auxiliary solvent.
25 per cent emulsion concentrate also containing 65 per cent of xylene and 10 per cent of Triton X-100.

Gamma Benzene Hexachloride:

An emulsion concentrate supplied by Canadian Industries Limited containing 4 per cent of gamma isomer.
A wettable powder containing 25 per cent of gamma isomer.
An oil solution containing 1.3 per cent W/V gamma isomer.

Parathion:

A wettable powder containing 25 per cent of active material.
A wettable powder containing 15 per cent of active material.
An emulsifiable concentrate containing 20 per cent of active material.

Methoxychlor:

A 50 per cent wettable powder prepared from a technical material containing 44 per cent of methoxychlor and 6 per cent of other isomers and reaction products.

Aldrin (Julius Hyman's Compound 118):

A wettable powder containing 25 per cent of active materials.
An emulsion concentrate containing 45 per cent of active materials.
10 per cent W/V solution in fuel oil with auxiliary solvent.
All of these were prepared from the technical compound containing 75 per cent of the active principle itself, and 25 per cent of related active compounds.

Dieldrin (Julius Hyman's Compound 497):

A wettable powder containing 15 per cent of active materials.
An emulsion concentrate containing 25 per cent of active materials.
5 per cent W/V solution in fuel oil with auxiliary solvent.
All of these were prepared from the technical compound containing 60 per cent of the active principle and 40 per cent of related compounds.

1, 2, 4-trichlorobenzene:

An emulsion concentrate containing 90 per cent of active material and 10 per cent of Triton X-100.

Except for one experiment, all exposure or application times were 15 minutes, so that the concentration in parts per million is obtained by dividing the dosage figures given in p.p.m.-minutes in the following tables by 15. Calculation of concentration was based on the percentage of the technical material in the formulation employed, except that for benzene hexachloride it was based on the percentage of gamma isomer.

The trichlorobenzene preparation was tested in 1949, in the hope that it might throw some light on anomalous results previously obtained with benzene hexachloride. 1, 2, 4-trichlorobenzene is an important breakdown product of benzene hexachloride (6).

Some difficulty was experienced in obtaining satisfactory emulsions and suspensions with the formulations of dieldrin, a stubbornly hydrofuge material. With the possible exception of methoxychlor, none of the wettable powders suspended well enough in the stream water in which they were used to permit their application with the dispenser described in the appendix to this paper.

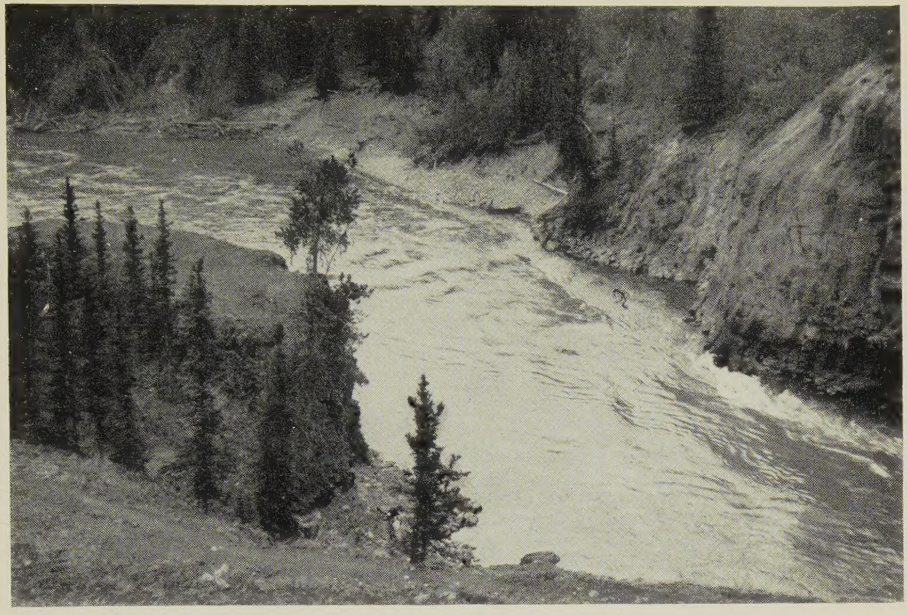

FIGURE 1. Miles Canyon, 6 miles south of Whitehorse, Y.T., on the Lewes River, treated with DDT in 1948. Photographed in 1949.

FIGURE 2. Prototype dispenser applying aldrin emulsion in experiment 6.

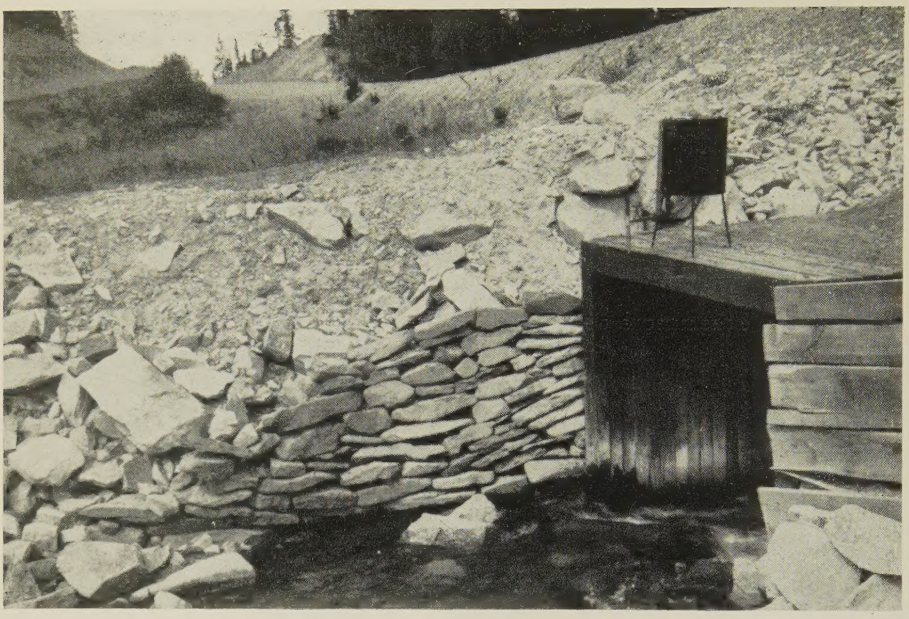

FIGURE 3. Modified dispenser applying DDT in fuel oil from a road culvert. Experiment 27.

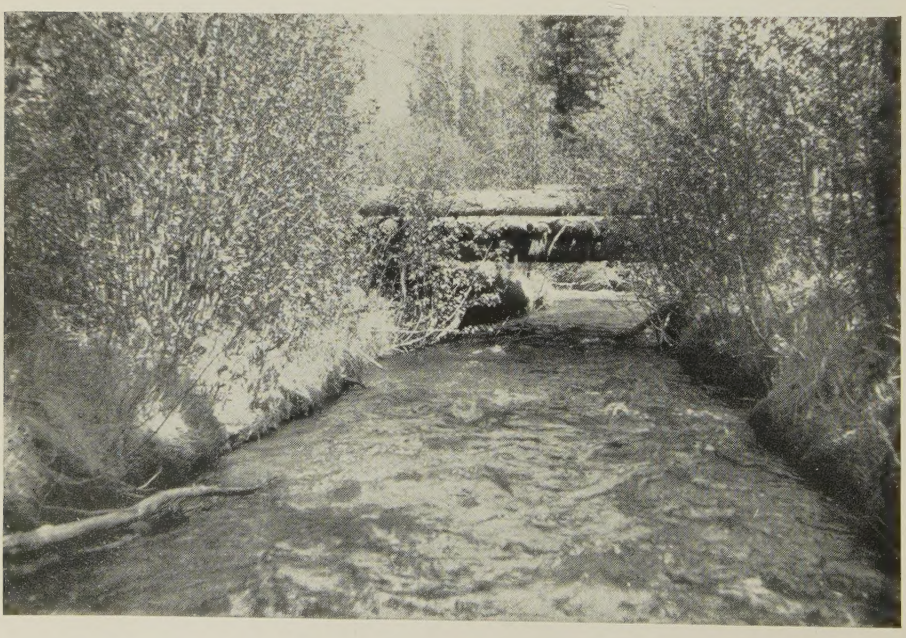

FIGURE 4. The treated stretch of Stony Creek, experiment 8.

OVICIDE EXPERIMENTS

Tests of the toxicity of these insecticides to black fly eggs were severely limited by the difficulty of obtaining eggs in suitable quantity and situation. When egg masses were found they were usually located at the upper end of a stretch of stream suitable for black fly breeding, so that it was not possible to divide the stream into a stretch for treatment and an upstream stretch for a check. Eggs located in this situation at Churchill in 1948, in all probability those of *Simulium venustum*, were taken into the laboratory for small-scale tests.

All the eggs used in each test were from a single egg mass, and probably of comparable age. Parathion suspensions and benzene hexachloride emulsions were tested on these eggs. Batches, consisting of several hundred eggs on the vegetation on which they were laid, were immersed for 15 minutes in dilutions of the insecticides prepared with stream water, and stirred gently during the tests. The eggs were then rinsed off with plain stream water and placed in tubes plugged with wet cotton. Check batches were similarly treated in plain stream water. Counts of the percentage hatch were made when hatching was complete in the check batches, and were confirmed 3 days later. The results are given in Table 1.

TABLE 1.—TOXICITY OF GAMMA BHC AND PARATHION TO BLACK FLY EGGS

Insecticide	Concentration p.p.m.	Dosage p.p.m.-min.	Percentage hatch
Parathion	0.2	3.0	80
	0.5	7.5	2
	1.0	15.0	0.3
Check 1 (untreated)	—	—	88
Gamma BHC	0.2	3.0	83
	0.5	7.5	15
	1.0	15.0	4
Check 2 (untreated)	—	—	99.5

No egg masses were found at Whitehorse in 1949, so that no further ovicidal data could be obtained. This may have been because it was too early in the season, or possibly because, as Smart (9) has suggested for *Prosimulium hirtipes*, the commoner species there may lay their eggs singly.

LARVICIDE EXPERIMENTS

The methods of test employed were virtually the same as those already described (5). Two additional methods of measuring the flow of streams were used, a Gurley flow meter, and a pitot tube with S.A.E. 10 oil as the manometric fluid. The float method which was used in 1947 was checked against the Gurley flow meter, and gave figures which agreed within 5 per cent. The pitot tube was also calibrated against the flow meter, and gave less consistent results; this equipment is cheaper and less cumbersome than the flow meter, however, and as the method is quicker it may prove suitable for use in routine control.

TABLE 2.—SUMMARY OF DATA FROM TREATMENT OF STREAMS NEAR CHURCHILL, MANITOBA, WITH PARATHION AND DDT AGAINST LARVAE OF BLACK FLIES, 1948

Experiment no. and date of treatment	Observed treated length of stream in yards	Average flow in cusecs	Temperature, °F.	pH	Insecticide formulation	Method of application	Dosage p.p.m./min.	Larval population		% emergence from pupae	
								Before treatment	24 hr. after treatment	Before treatment	After treatment
1. July 7	200	0.62	62	7.5	15% Parathion wettable powder	Medicine dropper	3	Moderate	Nil	67	22
2. July 22	300	0.33	64	8.0	15% Parathion wettable powder	Graduate and paddle	0.5	Light	Light	67	73
3. July 22	1400	30.8	62	7.5	10% DDT in fuel oil	Graduate	1.7	Moderate	Nil	—	—
4. July 24	5300	141	66.5	7.5	10% DDT in fuel oil	Graduate	1.04	Light	Nil	—	—

Experiments in 1948

Conditions at Churchill in 1948 were drier than in 1947, and this, together with the earlier use of considerable areas of country for experimental spraying with DDT from the air, prevented extensive work with insecticides in streams. The results obtained with DDT in 1947 were, however, confirmed by applications to Eastern Creek, on the same stretch as that employed in 1947; and to Herriot Creek, a well defined channel with substantial flow, entering the Churchill River from the west, some 25 miles south of Churchill. A length of nearly 3 miles of Herriot Creek was under observation.

Two preliminary tests of a 15 per cent parathion wettable powder were made, one in a small stream draining into a lake on the tundra east of Churchill camp, and one in Middle Eastern Creek. The results obtained in these four tests are given in Table 2.

At Whitehorse, in July, 1948, van Steenburgh and Twinn organized and made observations on a practical application of DDT in oil solution in the Lewes River. In an unpublished report, Twinn has stated as follows:

"The Lewes River was examined at several points from Marsh Lake northward to Whitehorse, a distance of about 20 miles. Throughout this length it flows swiftly and was found to be supporting an enormous population of black fly larvae and pupae, the larvae apparently predominating. Pupae taken from aquatic grasses on the rocks, in the shallow margin of the tumultuous rapids in the canyon south of Whitehorse, were *Simulium arcticum* Mall. Pupae of this species and *S. venustum* Say, and ones with 4 and 8 filaments in the respiratory tufts, probably *S. pugetense* D. & S. (=*Eusimulium costatum* Fried.) and *S. ottawaense* Twinn (=*S. decorum* Wlk.), were found in the river at points south of the canyon.....

"The site chosen for applying the insecticide was a weir or dam north of Marsh Lake, about 20 miles south of Whitehorse .... The drums of 5% DDT (in kerosene) were ... rolled on to the dam and spaced at equal distances across the river. Rough measurements at this point indicated the river to be 375 ft. wide, 10 ft. deep and flowing at 5+ ft. per second .... The calculated flow was 17,625 cu. ft. per second ... The temperature of the water was 62°F.

"When all was ready, on a signal the small bungs were removed simultaneously from the five drums, and the large bungs loosened. The DDT-oil solution poured fairly evenly into the river and all drums emptied themselves in 10 minutes, giving an actual dosage of 1 : 7, 135,000 at the point of application for that period (1.4 p.p.m.-minutes).

"The next morning, fourteen hours later, ... several known infested areas were re-examined along the margins of the rapids in the canyon about 15 miles below the point of application and also in Whitehorse about 20 miles therefrom. All larvae had disappeared from grasses and vegetation in the swift water, and only the pupae remained."

This was the largest river that had been successfully treated by ground application in Canada up to that time. The canyon referred to in this account is illustrated in Figure 1.

Experiments at Whitehorse in 1949

Most of this work was done in the area between Carcross and Whitehorse, Y.T., where a great number of streams in which black flies breed are readily accessible for survey and treatment, from the North West Highway System. Little knowledge of the fauna of these streams existed, and it was found during these experiments that the populations of species important as biters of man were relatively small; several species new to science were found. The work was done rather early in the season, which was very dry, the flow in the Lewes River in July being only about one-tenth of that in 1948. This lack of knowledge concerning some of the species involved is probably of little significance in relation to larval control, since no specific differences in susceptibility to insecticides have yet shown up in this work.

TABLE 3.—SUMMARY OF DATA FROM TREATMENT OF STREAMS NEAR WHITEHORSE,

Expt. no.	Date treated	Observed treated length in yards	Average flow in cusecs	Nature of bottom	Temp. °F.	Insecticide formulation
1	June 25	210	9.1	Fine gravel	40	497 emulsion concentrate
2	June 25	120	1.0	Gravel	42	497 emulsion concentrate
3	June 15	1000	1.4	Mud and sand	56	497 wettable powder
4	June 15	1500	1.7	Sand	40	497 wettable powder
5	June 29	250	2.5	Mud and rocks	45	497 in fuel oil
6	June 15	250	32.7	Rock and boulders	50	118 emulsion concentrate
7	June 15	150	0.58	Gravel	36	118 emulsion concentrage
8	June 15	800	14.7	Gravel and boulders	44	118 wettable powder
9	June 25	150	0.32	Sand and gravel	46	118 wettable powder
10	June 27	2000	6.6	Mud and sand	40	118 in fuel oil
11	June 15	100	1.0	Mud	42	Methoxychlor wettable powder
12	June 15	350	41.7	Mud and sand	54	Methoxychlor wettable powder
13	June 15	100	2.2	Gravel	38	Trichlorobenzene
14	June 15	100	0.39	Gravel and stones	38	Trichlorobenzene
15	June 25	120	1.3	Mud and sand	54	Trichlorobenzene
16	June 18	800	23.2	Mud and sand	53	gamma BHC emulsion
17	June 18	150	2.3	Mud and sand	41	gamma BHC emulsion
18	June 27	180	10.5	Sand	42	BHC wettable powder
19	June 23	50	7.5	Sand and gravel	38	BHC wettable powder
20	June 25	150	2.9	Sand	40	BHC in fuel oil
21	June 25	190	3.2	Gravel and boulders	40	BHC in fuel oil
22	June 23	200	1.8	Gravel	38	Parathion emulsion concentrate
23	June 23	150	1.4	Gravel and boulders	44	Parathion emulsion concentrate
24	June 25	320	0.87	Boulders	43	Parathion wettable powder
25	June 25	450	6.1	Sand and gravel	50	Parathion wettable powder
26	June 25	100	3.1	Gravel	42	10% DDT in fuel oil
27	June 25	250	4.6	Gravel	41	10% DDT in fuel oil
28	June 25	330	168	Rock and boulders	48	10% DDT in fuel oil

Y.T., WITH VARIOUS INSECTICIDES AGAINST LARVAE OF BLACK FLIES, 1949

Method of application	Dosage in p.p.m./min.	Larval control	Pupal control	Species present
Graduate	3	Excellent	—	*P. hirtipes*
Graduate	7.5	Good		
Graduate	3	Slight		
Graduate	7.5	Moderate	Doubtful	*Prosimulium* "J" and *P. hirtipes*
Eye dropper	15	Good		*S. arcticum*
Dispenser	3	Slight		
Dispenser	7.5	Slight		
Graduate	3	Slight	Moderate	*Prosimulium* "J"
Graduate	7.3	Moderate	Nil	*P. onychodactylum* D. & S. *Cnephia* "G" *P decemarticulatum* Twinn
Eye dropper	15	Slight		*P. hirtipes* and *E. costatum*
Graduate	3	Nil		
Graduate	7.5	Slight	Nil	*E. costatum*
Graduate	4.1	Nil		
Graduate	10.8	Nil		
Graduate	30	Nil	Good	*S. perissum* and *E. latipes*
Dispenser	7.5	Slight	Slight	*Cnephia* "D", *S. arcticum*
Dispenser	15	Slight	Nil	*E. costatum* and *P. hirtipes*
Graduate	3	Good		
Graduate	7.5	Good	Slight	*P. hirtipes* and *E. costatum*
Eye dropper	1.5	Good	*	*P. hirtipes*
Eye dropper	3	Good		
Graduate	1.5	Slight	—	*P. hirtipes*
Graduate	7.5	Moderate	Good	*P. hirtipes* and *E. costatum*
Graduate	7.5	Slight	—	*P. hirtipes*
Graduate	15	Excellent	Moderate	*P. hirtipes*
Dispenser	1.5	Excellent	Nil	*P. hirtipes* and *E. costatum*
Dispenser	1.5	Good		
Dispenser	1.5	Not assessed		*S. arcticum*

* % emergence greater in the treated than in the check batch; see explanation in section on toxicity to pupae.

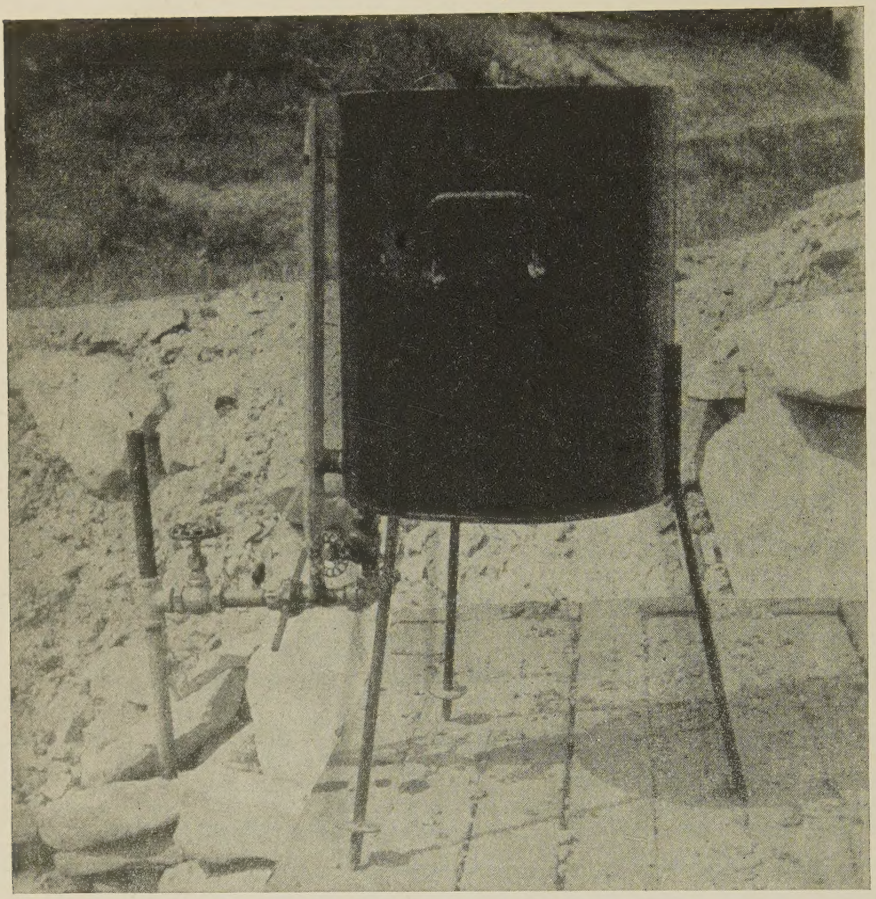

FIGURE 5. Close-up of modified dispenser applying DDT in fuel oil in experiment 27.

A new method of application was used in many of these tests, involving gravity flow from a tank fitted with a globe valve and a manometer connected across an orifice plate. Several changes were made in this apparatus during the course of the experiments, some of which are illustrated in Figures 2 and 4. In the appendix the apparatus is described in its final form, together with simplified instructions for its use in routine control measures.

The results obtained in these experiments are summarized in Table 3. Observations were made on the pH and salinity of all streams, and on the oxygen content of the water from the first 14 streams treated. Oxygen determinations were made by Winkler's method, and were discontinued when it was found that nearly all readings fell between 89 and 94 per cent of saturation. Readings were taken, however, for any stream which appeared exceptional, and in experiment 15, 100 per cent saturation was recorded, whereas that in experiment 20 was only 61 per cent. With the exception of experiment 2 (7.85) and experiment 5 (7.5), all streams had pH values between 8.15 and 8.75, measured on a Beckman pH meter. In all streams the salinity of the water, estimated by a nephelometric method, was less than 1 part of chlorides per million.

Specific identifications were made by G.E. Shewell of Systematic Entomology, Division of Entomology, Ottawa, for the most part from rather fragmentary material of samples taken to assess toxicity to pupae. Where no pupal sample was secured, specific identification has not, as yet, been possible, but there is good reason to suppose that the two commoner species, *Eusimulium costatum* Fried. and *Prosimulium hirtipes* Fries, were present in most of the smaller streams, and *Simulium arcticum* Mall. in the rivers. The occasional discovery of mature larvae with 6 filaments in the histoblasts of the pupal respiratory structures, together with earlier observations on the fauna in this area, suggest that larvae of the important biting species *Simulium venustum* Say may have composed a significant proportion of the populations actually killed by the treatments. Although some emergence of adults of *P. hirtipes* had already taken place when this work was started, this species did not live up to its reputation, and no observations were made of its biting man. Shewell's notes on the other species are given below with the accounts of the streams in which they were found.

Eight of the larger streams treated were identified by map names: Leguiel Creek (expt. 5), Stony Creek (expt. 8), Porter Creek (expt. 10), Little River (expt. 12), Judas Creek (expt. 16), McIntyre Creek (expt. 18), Robinson Creek (expt. 25), and Watson River (expt. 28). The remaining smaller streams were identified by milepost numbers of their crossings of the Alaska Highway, or of side roads. In addition to the 28 streams that were treated, 20 streams were surveyed and showed promise of being suitable for experimental treatment later in the season. Some typical streams are illustrated in Figures 2, 3, 5, and 6.

The following streams showed features of special interest:

Stony Creek (Expt. 8) and Stream No. 4.—Both of these streams flow into the Takhini River to the northwest of Whitehorse, the former on the left bank, about 30 miles above its efflux into the Lewes River (Figure 5), and the latter on the right bank about 20 miles above the efflux, crossing the Alaska Highway at mile 939.2. These were the only streams from which the species *Prosimulium* "J", apparently an undescribed species, was taken. They differ markedly in character: Stony Creek being swift, clean, and appropriately named; No. 4, sandy, sluggish, and cloudy.

Experiment No. 9.—This little stream was the only source of specimens of *P. onychodactylum* D. & S. and of a new species, *Cnephia* "G". The pupal filaments of the latter resemble the antlers of caribou. The stream crosses the Carcross Road at Milepost 16, draining from a swamp just to the east of it, and pursues a somewhat vague course through dense vegetation and deadfall.

Experiment No. 15.—Two species, *S. perissum* D. & S. and *Eusimulium latipes* (Mgn.), were recorded from this stream and nowhere else in the locality. The former was more abundant than any other species in any other stream; the latter was represented by a single empty pupal skin. The physical characteristics of the stream were also unusual. It is a small, smooth, slow-flowing, warm stream, winding across an old lake-bed. It was one of the few streams with much aquatic vegetation, and the only one in which the oxygen content of the water reached saturation. The stream crosses the Carcross-Tagish highway 9.5 miles from its junction with the Alaska Highway.

Judas Creek (*Expt. 16*).—*Cnephia* "D", a species closely related to *C. boreale* (Mall.), was taken from this stream only; it was common there in association with *Simulium arcticum*. The larvae in this stream reacted to the benzene hexachloride emulsion in a characteristic manner, many releasing on threads before the end of the treatment, but control was incomplete and reinfestation rapid. Judas Creek crosses the Alaska Highway at mile 872.1.

Experiment No. 20.—P. hirtipes was found in this stream, which crosses the Carcross-Tagish highway 22.2 miles from its junction with the Alaska Highway.

Watson River (*Expt. 28*).—By treating this river with DDT at a point west of Robinson, on the Carcross highway, and making an assessment at its efflux into Lake Bennett, it was hoped to secure observations on the effect 20 miles below the application point. The river, however, proved inaccessible at the latter point.

When no living larvae could be discovered in the observed length of stream after treatment, this was recorded as excellent control; control was recorded as "nil" if no change in the population could be detected. If scattered larvae were found throughout the observed length, or if the distance for which the larvae were eliminated or killed was less than the observed length, control was classed as slight, moderate, or good, according to the degree of infestation remaining in comparison with the initial population. Treatments were assessed as nearly as possible 24 hours, and always between 18 and 30 hours, after application.

TOXICITY TO PUPAE

For insecticides applied to streams the only simple method for estimating the toxicity to the pupal stages of black flies is to take samples of pupae before and after treatment, and record the percentage of adult emergence from each sample in the laboratory. The second pupal sample was usually taken 24 hours after treatment, since the stream was revisited for assessing larval mortality after this interval and since it was desirable to allow adequate time for the "tail" of insecticide to pass. This method is reasonably satisfactory with species such as *Simulium venustum* and *S. perissum*, which give a high and predictable emergence under laboratory conditions; but it was not satisfactory with species such as *Prosimulium hirtipes*, which did not emerge in tubes plugged with wet cotton.

For this reason, the more valuable results on this question have been obtained at Churchill, where *S. venustum* is the dominant species. Results obtained in 1948 in conjunction with tests against larvae are recorded in Table 2, and other data obtained at Churchill in 1948 and 1949 are summarized in Table 4. Such limited data as were obtained at Whitehorse in 1949 are given in Table 3; a blank space in the pupal control column indicates that no pupal samples were obtained; a dash indicates that samples were secured but that no emergence took place from either treated or untreated pupae.

Observations during the course of this work have shown clearly that the resistance of black fly pupae to most insecticides increases sharply with age. The same appears to apply to the resistance to exposure to the air;

FIGURE 6. Part of the stream treated in experiment 6, downstream from that in Figure 2.

FIGURE 7. The stream treated in experiment 13.

FIGURE 10. Typical black fly larvae (after Twinn).

FIGURE 11. Typical black fly pupae (after Twinn).

TABLE 4.—SUMMARY OF DATA ON TOXICITY OF BENZENE HEXACHLORIDE AND TRICHLOROBENZENE TO PUPAE OF *Simulium venustum*, CHURCHILL, MANITOBA, 1948 AND 1949

Date	Insecticide formulation	Dosage p.p.m./min.	No. of pupae	% Emergence	
				Before treatment	After treatment
Aug. 5, 1948	BHC oil solution	1.8	2× 100	26	44
Aug. 5, 1948	BHC oil solution	3.6	2 × 100	42	48
Aug. 5, 1948	BHC oil solution	3.6	2 × 100	29	80
Aug. 5, 1948	BHC oil solution	7.2	2 × 100	9	80
Aug. 4, 1949	1, 2, 4-trichlorobenzene	15	2 × 50	78	84
Aug. 4, 1949	1, 2, 4-trichlorobenzene	30	2 × 50	78	88
Aug. 11, 1949	1, 2, 4-trichlorobenzene	60*	2 × 25	92	68

* Test conducted in water from the stream.

the greater the average age of a sample of pupae, the greater the percentage emergence of adults in tubes plugged with wet cotton. A random sample of pupae taken 24 hours after the successful application of a larvicide is of greater average age than a check sample taken before treatment or from an untreated part of the stream. The amount of this difference in average age depends on the frequency distribution of age groups; at the commencement of pupation it approaches its maximum possible value of 24 hours, and may have a significant effect on the percentage emergence. In spite of this effect, however, there is evidence that treatment with benzene hexachloride in oil solution has a stimulating effect on the emergence of the adult, although benzene hexachloride emulsions (5) and suspension effect a measure of control.

EFFECTS ON OTHER ORGANISMS

The influence of insecticidal treatments of streams on other organisms may be of considerable importance in any one of three main ways. Organisms utilized as food by black fly larvae may be affected. Apart from a brief reference (7) to the difficulty of sectioning these larvae on account of the siliceous skeletons of diatoms in the alimentary canal, and an account by Smart (10) indicating how catholic their tastes may be, published information on the nature of these organisms is scarce. Of greater importance is the possible effect on the predators of black fly larvae, either directly or through other organisms used as food. Although many insects, in particular predatory larvae of Trichoptera, come in this category, probably the most important organisms are fish. Many species of fish feed readily on black fly larvae; 71 larvae of assorted age have been taken from the stomach of a northern sucker, *Catostomus catostomus* Forster, captured in a sparsely infested creek, and 73 very young larvae from the stomach of a nine-spined

stickleback, *Pungitius pungitius* Linnaeus, from a stream which observers had reported to be free from infestation. Larvae have also been found in the stomachs of jackfish, *Esox lucius* (L.), and of the northern wood frog, *Rana sylvatica cantabrigensis* Baird. Finally, and perhaps of the greatest immediate importance, is the possible influence on other animals, particularly fish, of economic value. This, too, of course may be a direct influence at any stage of the life-history, or an indirect influence through the food supply.

Toxicity to Fish

At Churchill in 1948 a number of tests were made by P. M. Jackin, of the Defence Research Board, of the effects of three of the more important insecticides, DDT and BHC emulsion concentrates and parathion wettable powder, on local species of fish in tanks and cages. The species of fish used were: jackfish, *Esox lucius:* northern sucker, *Catostomus catostomus:* lake trout, *Cristivomer mamaycush* Walbaum; arctic grayling, *Thymallus signifer* Richardson; brook stickleback, *Eucalia inconstans* Kirtland; and nine-spined stickleback, *Pungitius pungitius:* the last two were treated collectively. All exposures were for 15 minutes. Although the numbers of fish used were rather small, the following tentative conclusions from the results reported* are justified:

Jackfish, suckers, and lake trout did not suffer any visible or permanent injury as adults from single dosages of DDT up to 600 p.p.m.-minutes, 400 times that required for control of black fly larvae; 50 per cent mortality of sticklebacks occurs at about 150 p.p.m.-minutes, and of fry of arctic grayling at about 450 p.p.m.-minutes; sucker fry show no immediate symptoms after 150 p.p.m.-minutes.

Parathion did not kill adult suckers at 150 p.p.m.-minutes, and there was no immediate effect on fry at 250 p.p.m.-minutes. Fifty per cent mortality of sticklebacks occurred at about 900 p.p.m.-minutes.

Gamma BHC had no effect on the fry of suckers at 1.5 p.p.m.-minutes but these became violently active after 6.7 p.p.m.-minutes; 50 per cent mortality of both sticklebacks and of grayling fry occurred at about 20 p.p.m.-minutes.

After treatment with DDT at about 100 p.p.m.-minutes, jackfish began to open and close the jaws rapidly and then to swim violently about the tank. Continued exposure resulted in a comatose condition and in some interference with orientation mechanisms; on being returned to fresh water, however, most of the fish recovered overnight.

Toxicity to other arthropods

As in 1947, samples of the organisms killed or incapacitated in stream treatments were taken by means of screens fixed in the streams at the lower end of the observed stretch. At least one such sample was taken for each of the newly tested active materials. Finer screens (40 mesh) were used than in 1947, and in 1949, an attempt was made to introduce a quantitative element into this phase of the work, screens 6 inches square being used, and the flow through these being measured at the beginning and end of the experiment.

* Entomological research in northern Canada. Progress Report, 1948, Pt. 2. Def. Res. Bd. Rept. D.R. 19. 1949.

In larvicide experiment 1 at Churchill in 1948 (Table 2), the first test with parathion, a large screen was used; and in a portion of the material from this screen there were the following numbers of insects and other arthropods:

MOLLUSCA		
Gastropoda		
Pulmonata		3
ARACHNIDA		
Acarina		
Hydrachnidae		3
CRUSTACEA		
Branchiopoda		59
Copepoda		22
INSECTA		
Apterygota		
Collembola		
Arthropleona		8
Expoterygota		
Plecoptera		
Nemouridae	nymphs	87
Ephemerida	nymphs	21
Hemiptera		
Corixidae	nymphs	1
Endopterygota		
Coleoptera		
Dytiscidae		3
Trichoptera	larvae	2
Diptera		
Nematocera		
Chironomidae	larvae	5
	pupae	3
	adults	6
Heleidae		
Culicoides sp.		2
Tipulidae		1
Culicidae		
Aedes sp.		1
Simuliidae	larvae	323
	pupae	5
Mycetophilidae		2

In so far as it was possible to determine from a field examination, the crustaceans were all alive at the time of collection, 24 hours after treatment; the insects, however, were dead.

The results of an examination of the quantitative screen samples obtained in 1949 at Whitehorse are summarized in Table 5. With the exception of mature larvae of Simuliidae, specific identification has not been possible. All sufficiently mature black fly larvae were identified to species, but the results did not suggest specific variation in susceptibility to any insecticide. The arachnids were all mites of the family Hydrachnidae, representing 3 species. The Collembola represented 2 genera, *Proisotoma* in the Isotomidae, and *Smynthurus*. Three genera of stoneflies were represented, *Isoperla*, *Alloperla*, and *Nemoura*, there being several species of the last. The mayflies belonged in either the Baetidae or the Ecdyuriidae. The caddis flies showed the greatest diversity for the number of specimens obtained, the following groups being represented; Brachycentridae: *Micrasema*, 2 species; Limnephilidae: 2 species; Rhyacophilidae: *Rhyacophila vibox* Milne; Phryganeidae and Psychomyidae: one species each.

TABLE 5.—THE EFFECTS ON INSECTS AND ARACHNIDS OF TREATMENT OF STREAMS WITH VARIOUS INSECTICIDES, WHITEHORSE, Y.T., 1949

Experiment No.	4	6	8	12	14	16	17	19	22
Insecticide	Dieldrin (wettable powder)	Aldrin (emulsion concentrate)	Aldrin (wettable powder)	Methoxychlor	Trichloro-benzene	BHC (emulsion concentrate)	BHC (emulsion concentrate)	BHC (wettable powder)	Parathion (emulsion concentrate)
Dosage, p.p.m.-min.	7.5	3.0	3.0	7.5	10.8	7.5	15.0	7.5	1.5
Approximate volume of water sampled (1,000 cu. ft.)	19.4	75.0	39.0	13.1	18.0	38.3	17.0	26.0	21.6
No. of specimens taken, dead/alive									
Arachnida	0	0	0	0	2/1	6/1	0	0	0
Insecta									
Collembola	1	0	0	0	0	1	0	1	0
Plecoptera (nymphs)	419/2	4/1	5/2	12/1	0/2	134/6	7/4	7	32
Ephemerida (nymphs)	103/1	4	3/1	27/1	0	107	1	2	15
Trichoptera (larvae)	5	1	1	0/1	0	24/2	0	0	1
Diptera									
Chironomidae (larvae)	2/1	0	3	0/3	3	19/2	7	2/1	7
Chironomidae (pupae)	0	2	4	4	0	4/1	0	1	2
Simuliidae (larvae)	19/1	3/1	4/1	7	0	94/5	5/1	1	40/7

Other groups, not shown in Table 5, were represented by single specimens; these included 2 crustaceans, a species of cyclops and an ostracod; corixid nymphs; other nematocerous larvae; and many specimens obviously not from the aquatic habitat, such as numerous thrips, psyllids, and mycetophilid and lonchopterid flies. The most striking differences between these samples and the samples previously obtained at Churchill were the small amount of material obtained and the virtual absence of Crustacea and Collembola. The very low chloride content of the water may be the principal factor responsible for these differences.

In view of the very varied nature of the streams, comparative deductions from these data are scarcely possible. It appears, however, that none of these insecticides is usefully selective in toxicity to black fly larvae, although crustaceans are apparently resistant to parathion, and 1, 2, 4-trichlorobenzene has a lower general toxicity than any of the other materials.

DISCUSSION

Parathion wettable powder and benzene hexachloride emulsion each have useful ovicidal properties, at least against eggs believed to be those of *Simulium venustum*; but until more is known concerning the oviposition sites and egg-laying habits of other species, it is doubtful whether much practical use can be made of this. F. J. H. Fredeen, in studies of *S. arcticum* in the South Saskatchewan River, has recently shown that eggs of this species are to be found buried in the sand of the stream bed; other species may oviposit above water level.

For controlling the larvae of the great majority of the important Canadian species of black flies, none of the new materials tested compares at all favourably with DDT in oil solution. All the formulations of dieldrin and parathion that were tested, and the wettable powder and oil solutions of benzene hexachloride, give control, at least of certain species, at dosages not much greater than that required for DDT; but none of these exhibits any encouraging selectivity in its effects on stream fauna.

Some toxicity to the pupae of certain species is shown by aldrin wettable powder, by benzene hexachloride and parathion emulsions and wettable powders, and by trichlorobenzene at high dosages. This toxicity, however, decreases rapidly with increasing pupal age, until by the time the pupa is surrounded by an air film it can obviously be affected only by fumigant activity such as that of trichlorobenzene. In practice, it is again doubtful whether any use can be made of these properties, except in special circumstances. If a population has been allowed to develop until a significant proportion has pupated, it might be a satisfactory procedure to spray the vegetation bordering the stream with a DDT emulsion at first emergence to kill adults.

At first sight the results of tests against grown fish are encouraging; newly hatched fry, however, are likely to be far more susceptible, and interference with spawning is a possibility. It is clear that the long-term effects on the arthropod fauna of repeated treatments of streams with any of these chemicals except perhaps trichlorobenzene are likely to be profound. To what extent these effects would influence fish is impossible to predict; a long-term ecological survey of a large stream under such repeated treatments would be needed to determine this point. The statement of Travis (11) that: "Studies. . . . showed that DDT caused no damage to fish or fish foods at 30 to 60 p.p.m." is, however, at variance with findings reported in 1949 (5) and in this paper, unless the exposure time in the studies in question was reduced to the rather impracticable figure of about 1.5 seconds.

It is difficult to estimate the effect which local killing of black fly larvae would have on the human pest problem in an area. This would depend principally, of course, on flight range and flight habits of the species concerned. Though the flight range of *S. arcticum* is known to be considerable, there are some grounds for believing that for many important pests of man the range is much more limited. This question can best be settled by a double approach: direct observation on the flight habits of the species concerned, and field trials of large-scale area control in the larval stage coupled with periodical assessments of adult populations.

Two steps, a long-term ecological study of the influence on the fauna of repeated treatments of streams with DDT, and the accumulation of data on flight range and flight habits of important biting species, are of paramount and immediate importance in the progress of black fly control work.

SUMMARY

Various formulations of DDT, parathion, gamma benzene hexachloride, methoxychlor, aldrin, dieldrin, and 1, 2, 4-trichlorobenzene were tested in the field for toxicity to the larvae and pupae of several species of black flies (Simuliidae). Laboratory tests of parathion suspension and benzene hexa-

chloride emulsion showed that these materials have some ovicidal properties. Against larvae, DDT in oil solution is still by far the most satisfactory insecticide. Parathion; benzene hexachloride; aldrin; and trichlorobenzene at high concentrations show some toxicity to pupae, but the practical value of this and of the ovicidal materials is doubted.

DDT, benzene hexachloride, and parathion were tested on the fry and adults of several species of fish. The absence of any immediate permanent effects at the dosages required to kill black fly larvae is encouraging.

Observations on the effects of all insecticides except DDT on other fauna in the streams treated are recorded. The results do not suggest that the toxicity of any of the materials is more selective to black fly larvae than that of DDT.

A dispenser for the convenient and accurate application of insecticides to streams is described in an appendix, and simplified instructions for its use are given.

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APPENDIX

INSTRUCTIONS FOR THE CONTROL OF BLACK FLIES

Black flies can be controlled by the application of an oil solution of DDT, preferably 10 per cent, to streams in which the larvae (Figure 10) develop. Since these solutions affect many other insects and animals, including fish, it is important that the correct quantity be carefully applied at the proper time and place.

The quantity required is arrived at in the following way. At any convenient point measure the width (W) and average depth (D) of the stream in feet, and its maximum speed of flow (V) in feet per second. The speed can be obtained by timing a fairly large floating object over a measured distance, not less than 10 feet. The float used should not project much above the water, or wind will affect its movement; a piece of wet wood is suitable. If much work is to be done, a pitot tube or a hydrographic flow meter would be worth-while. The quantity of a 10 per cent solution required is then:—

$$17 \times VWD \text{ cc.}$$

This amount can be measured in a graduate, and dribbled into the stream by hand as evenly as possible over a period of 15 minutes. If much work is to be done, however, a dispenser constructed as shown in Figure 8 is useful.

The best time to make this application is immediately before the larvae transform into pupae (Figure 11); in practice the appearance of the first pupae should be carefully watched for, and application should then be made as soon as possible. It is best to apply the insecticide upstream of as much of the infestation as possible, preferably just above a turbulent area in order to improve mixing.

Construction and calibration of a dispenser

A suitable dispenser can be constructed almost entirely from a 10-gallon oil drum and standard pipe fittings. The essential features are the two valves, the two manometer tubes with scale, and provision for changing the orifice plates, which are held in the gasket-type union (L). Leather washers cemented to either side of these plates are a convenience, and a leather gasket under the lid reduces waste in transit. Various modifications of the design shown are possible, to take advantage of locally available material.

Before use, the dispenser requires calibration, preferably with the DDT solution for which it is to be used and at about the same temperature. The tank is filled with this solution strained through 50-mesh screening, and the first valve (N) is opened fully. The fluid then rises to the top of both manometer tubes. As the second valve is opened the level in the second tube falls progressively. It is necessary to measure the delivery rate in cc. per 15 minutes for various pressure differences in the two tubes, so that a curve connecting the two can be drawn as shown in Figure 9. This must be done for each of the three orifice plates.

INSECTICIDE DISPENSER FOR STREAM TREATMENT

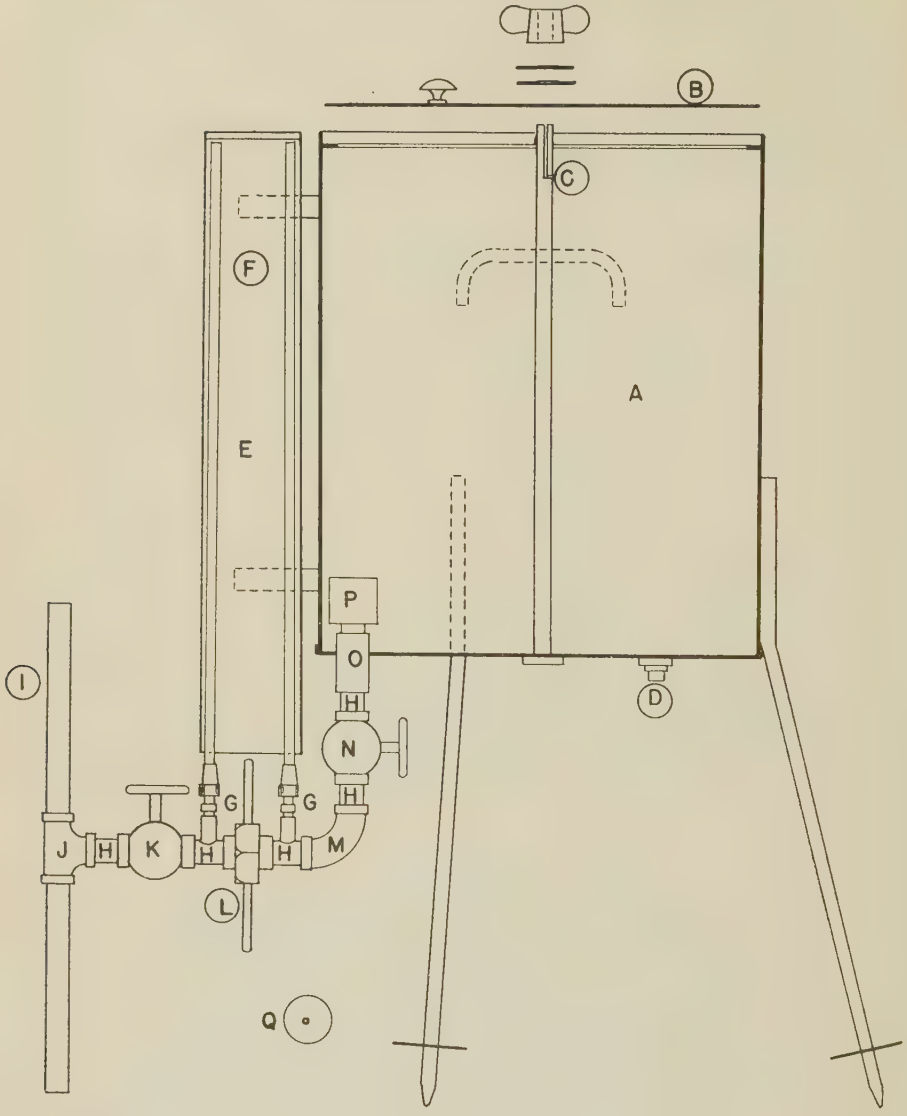

FIGURE 8. Insecticide dispenser for stream treatment. Construction drawing for final design.

A. 10-gallon drum
B. Lid, 1/16″ steel
C. ½″ bolt with air vent
D. Drain plug
E. Centimeter scale board
F. ¼″ Saran tubes (2)
G. ⅛″ I.P.S. × ¼″ Saran Adapter (2)
H. ½″ × 1½″ nipples (5), 2 with ⅛″ couplings welded on
I. ½″ × 7″ pipe (2)
J. ½″ std. tee
K. ½″ plug disc or needle point brass globe valve
L. ½″ gasket type union, 2″ lugs welded on,
M. ½″ std. elbow
N. ½″ composition disc brass globe valve
O. ½″ × 2″ coupling welded in
P. 50-mesh brass screen cylinder 1½″ × 1½″ soldered to ½″ nipple
Q. Brass orifice plate ⅛″ × 1½″ D. (3), drilled 0.020″, 3/64″, and 9/64″

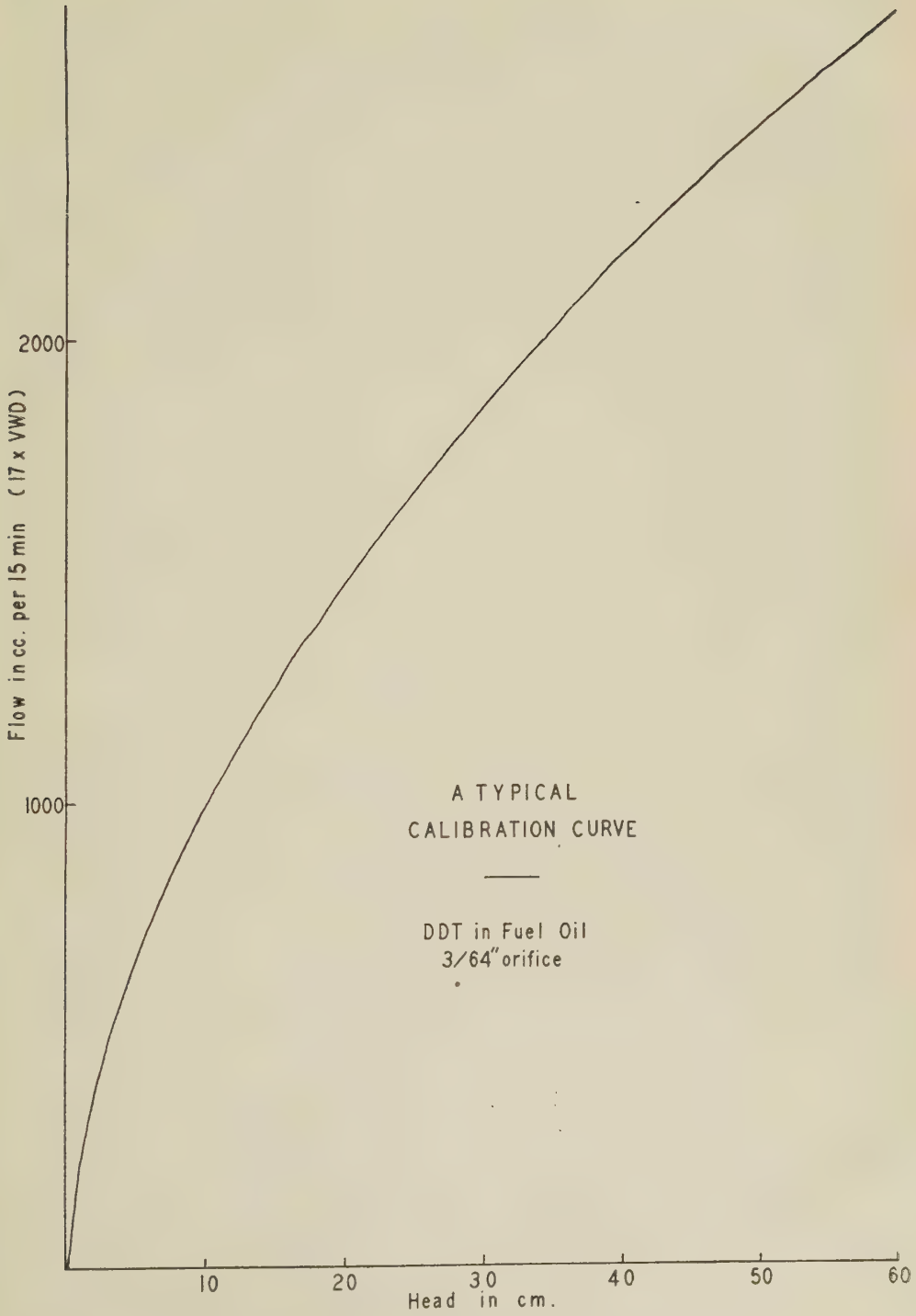

FIGURE 9. A typical calibration curve.

Use of dispenser

The dispenser may be set up on the bed of the stream, or on a bank, bridge, or culvert, so that the delivery pipe will discharge into the water. A short length of rubber hose, three-quarters of an inch in diameter, may be useful to conduct the insecticide. The tank should be levelled from front to rear. This can be done by raising or lowering the rear leg until both manometer pipes give the same reading. The appropriate orifice and pressure difference for any stream can be arrived at by looking up the figure 17 × VWD on the calibration curves. With the correct orifice in position, the second valve (K) is opened until the pressure difference reaches the required figure, and the insecticide is allowed to run for 15 minutes. In treating larger rivers, it may be necessary to adjust the valve K during the the 15 minutes to compensate for the fall in level in the tank.

Rivers with flow up to about 1,000 cubic feet per second can be conveniently treated with this equipment.

VARIATION IN THE SEED SETTING ABILITY OF LADINO WHITE CLOVER[1]

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Environmental conditions in Eastern Canada are not in general favourable to seed production of Ladino clover. Whether this condition is due to lack of adaptation of the plant or to unfavourable environment has not been determined. Nevertheless, one of the main objectives of the Ladino breeding programme instigated in 1947 was to increase seed productivity. At the same time data were recorded from the breeding material with the purpose of investigating whether the range of variation of some of the characters involved in seed productivity was large enough to justify selection.

Very little information is available on the genetical variability of white clover in relation to seed productivity. Differences in the abundance of blossoms were observed between strains by Ahlgren and Sprague (1). Atwood (2, 3) has reported that the number of seeds set in compatible crosses differed between plants. According to Erith (3), many ovules are never fertilized, the number of fully developed seeds in the legume varying from one to seven. The majority of the legumes contain only two or three seeds.

The purpose of this investigation was to determine the influence of climatic environment and genetical variability on seed setting and percentage of seed-bearing florets in Ladino white clover.

MATERIALS AND METHODS

The materials used throughout this investigation came from commercial seed sources. Individual plants were started in the greenhouse in April and transplanted to the field in May. Seedlings were set three feet apart on clay soil. Data were recorded during the first year of growth.

In general four heads of equal maturity were sampled from each plant on a given date. In some cases a random sample of 10 florets was taken from each head to determine seed setting. In other cases all florets were counted and the number of seeds recorded individually. In these investigations, whole heads were used, unless otherwise specified. These determinations were made under field conditions. Nothing was allowed to interfere with honey bee pollination. The farm apiary was located within one mile of the white clover nurseries.

EXPERIMENTAL RESULTS

Effect of environment

Ten spaced plants of Ladino clover were used to measure the effect of climatic environment on seed setting. They were selected among other plants of the 1947 breeding nurseries for their early and profuse flowering. Four heads, from which all but 10 blooming florets were removed, were

[1] Contribution from the Division of Forage Plants, Experimental Farm Service, Department of Agriculture, Ottawa, Canada.

[2] Agronomist, Forage Crops Breeding.

tagged every week on each of the 10 plants. In general, pollination occurred within a week, except during periods of cold weather. The mature heads were harvested 5 weeks later and the number of seeds was recorded for each individual floret. Storms and foliage density were responsible for the loss of several tagged heads, which could not be found. Some missing data were due to the lack of flower heads on various plants at certain dates. The results obtained are summarized in Table 1.

TABLE 1.—NUMBER OF SEEDS PER FLORET AND PERCENTAGE OF SEED-BEARING FLORETS IN RELATION TO THE MEAN VALUES OF SOME CLIMATIC FACTORS OBSERVED AT WEEKLY INTERVALS DURING THE SUMMER 1948

Items	Dates of flowering											
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	9/1	9/15
Number of plants	10	10	10	10	10	10	10	10	7	8	8	6
Number of heads	40	39	40	39	39	37	32	35	27	20	30	20
Number of flowers	337	369	387	396	363	328	293	340	232	188	283	194
Per cent seed-bearing florets	85.6	63.8	74.5	85.9	86.8	84.5	84.4	81.3	91.9	91.4	88.4	0.8
Number of seeds per flower	2.0	1.4	1.9	2.4	2.3	2.2	1.9	2.0	2.7	2.5	2.4	0.03
Weekly means of:	Beginning on:											
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	9/1	9/15
Max. temperature	77.4	74.8	79.8	79.4	74.8	80.1	69.7	75.1	79.6	77.8	79.7	60.8
Min. temperature	53.4	52.7	55.1	53.0	56.6	60.6	51.8	52.4	55.6	58.1	53.7	41.1
Rain duration (hours)	4.75	14.00	7.75	4.50	27.50	8.50	22.75	18.00	7.50	16.25	0.00	43.00
Rainfall (inches)	0.56	0.62	0.67	0.34	1.14	0.40	0.89	1.80	0.27	0.67	0.00	2.25
Sunshine (hours)	50.7	59.0	64.2	55.4	39.8	76.2	50.1	59.4	70.8	58.8	65.2	12.2
Bee activity (hours)	65	62	68	71	53	73	48	56	66	54	66	11

The average number of seeds per floret varied during the season from 0.03 to 2.7. Florets pollinated during the period from August 18 to September 1 gave the highest seed sets. Poor seed setting was obtained on the florets pollinated in the early part of July. Flowers blooming in the middle of September produced a small amount of aborted seeds. The very low yield of sound seeds is explained by the occurrence of a killing frost during the process of seed formation. A few seeds in one plant only were matured enough to escape frost damage. The results obtained on September 15 were excluded from the analysis of variance. Differences between the number of seeds per floret observed at different dates were highly significant, with a least significant difference of 0.4 at the 1 per cent level.

The percentage of seed-bearing florets varied from 0.8 per cent to 91.9 per cent according to the date of flowering. It was noted that a variable number of florets did not produce any seed at all dates, due apparently to a lack of pollination. The percentage of seed-bearing florets was particularly low in the early part of July; this explains the low seed setting obtained during that period.

The degree of pollination seems to be an important cause of variation in seed setting during the season. The weekly variations in seed setting are apparently a result of the complex interaction of several climatic factors. High temperatures, little rain of short duration and abundance of sunshine which are favourable to bee activities, tend to increase seed setting, although it is difficult to find any close relationship between seed setting and any climatic factors, with the exception perhaps of rain duration.

Distribution of the number of seeds per floret

A total of 91,481 individual florets were examined in 1948 and 1949 from a large number of plants among unselected populations of Ladino white clover. The frequency distribution for the number of seeds per floret is recorded in Table 2.

On the average 11 per cent of the florets did not produce any seed. The maximum number of seeds per floret was 7, with a frequency of less than 1 per cent. Two or three seeds per floret were the most frequent classes. There were slight changes in class frequencies from year to year and within a season. The greatest changes were observed between individual plants.

Variation between plants

The seed productivity of 131 individual plants of Ladino clover was studied at two different dates: July 8-12 and August 17-18, 1948. Seed setting was measured by two methods. One method consisted in retaining only 10 flowers on each head, at the stage where these flowers were just about to open. These heads were then tagged, and harvested 5 weeks later. In the other method, the whole head was tagged when the lower florets were starting to bloom, and they were harvested after they had reached maturity. Seed counts were made by dissecting each pod individually.

The mean number of seeds per floret was calculated by dividing the total number of seeds by the total number of flowers within each head, and by averaging the results from the four heads at both dates for both methods. Highly significant differences were observed between individual plants. The mean number of seeds per floret ranged from 0.8 to 4.3 with an average of 2.38 seeds per floret. The variation among plants is demonstrated graphically in Figure 1. Plant distribution was normal. From those 131 plants, 2 plants were found with a seed setting rating which is 4 standard deviations removed from the mean.

Significant differences were found in the seed setting values obtained with the two methods of measurement. There was a tendency to underestimate the number of seeds per floret when a sample of 10 florets was used instead of the whole head. The high degree of consistency between the two methods justifies the use of either one to estimate the number of seeds per floret, when comparative results are the main purpose. However preference should be given to the method using whole heads, if small differences are to be detected, because the variance and the coefficient of variability are appreciably lower with the latter method. Significant

TABLE 2.—FREQUENCY DISTRIBUTION OF FLORETS IN RESPECT TO NUMBER OF SEEDS AMONG UNSELECTED POPULATIONS OF LADINO WHITE CLOVER

Dates	Seeds per floret								Number of florets
	0	1	2	3	4	5	6	7	
July 1948	4,600 11.61%	6,198 15.64%	8,543 21.56%	8,815 22.24%	6,594 16.64%	3,173 8.01%	1,311 3.31%	393 0.99%	39,627 100.00%
August 1948	2,937 10.12%	4,810 16.58%	6,896 23.77%	7,209 24.84%	4,814 16.59%	1,786 6.16%	514 1.77%	49 0.17%	29,015 100.00%
1949	2,856 12.51%	3,712 16.25%	5,347 23.41%	5,644 24.71%	3,704 16.22%	1,267 5.55%	300 1.31%	9 0.04%	22,839 100.00%
Total	10,393 11.36%	14,720 16.09%	20,786 22.72%	21,668 23.69%	15,112 16.52%	6,226 6.81%	2,125 2.32%	451 0.49%	91,481 100.00%

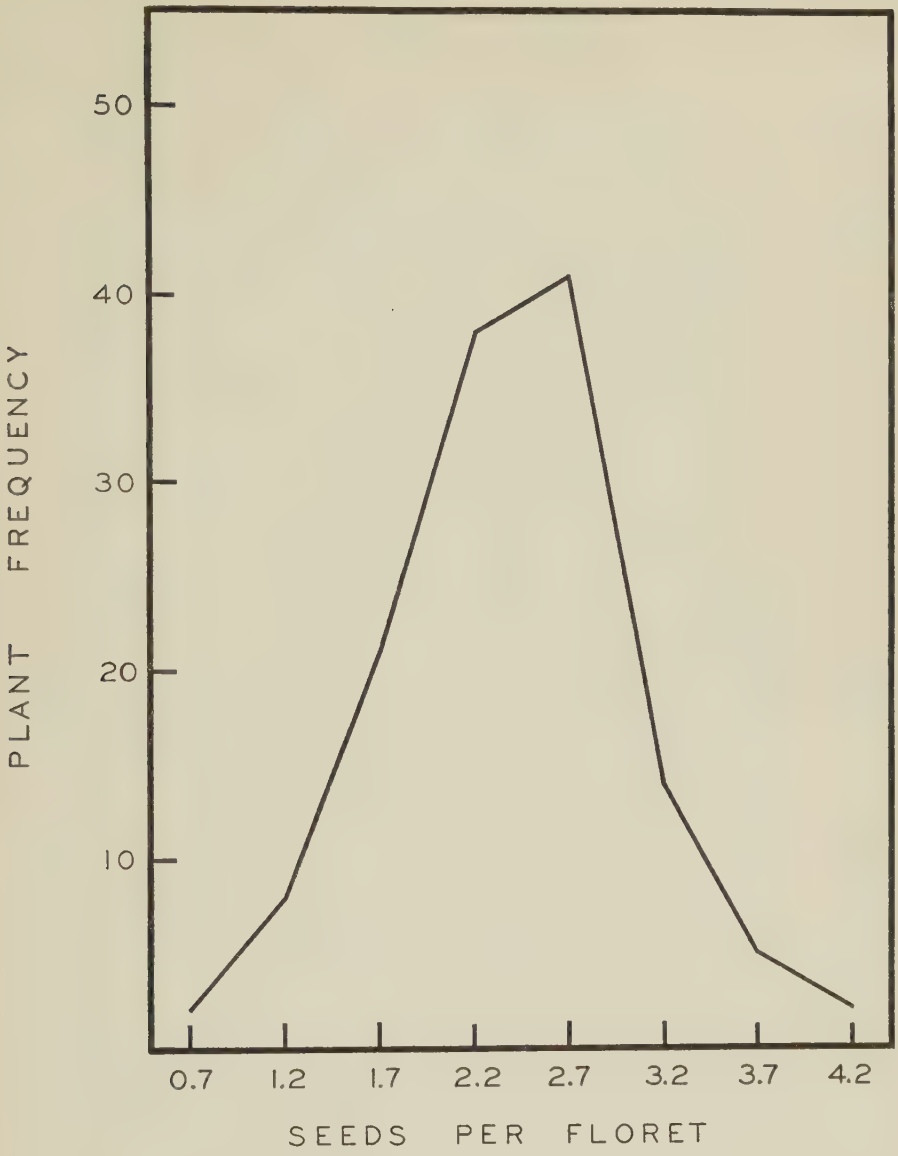

FIGURE 1. Frequency curve of the number of seeds per floret among 131 individual Ladino plants in 1948.

differences between the two dates indicate that a better seed setting was obtained from florets blooming in July than from those blooming in August. These results conflict with those reported in Table 1.

A seed setting determination was made in 1949 from 90 plants of a pure Ladino seed lot supplied by the Oregon Agricultural Experimental Station. The results are shown graphically in Figure 2. The mean number of seeds per floret ranged from 1.2 to 3.7, with an average of 2.5. Differences between plants were highly signficant.

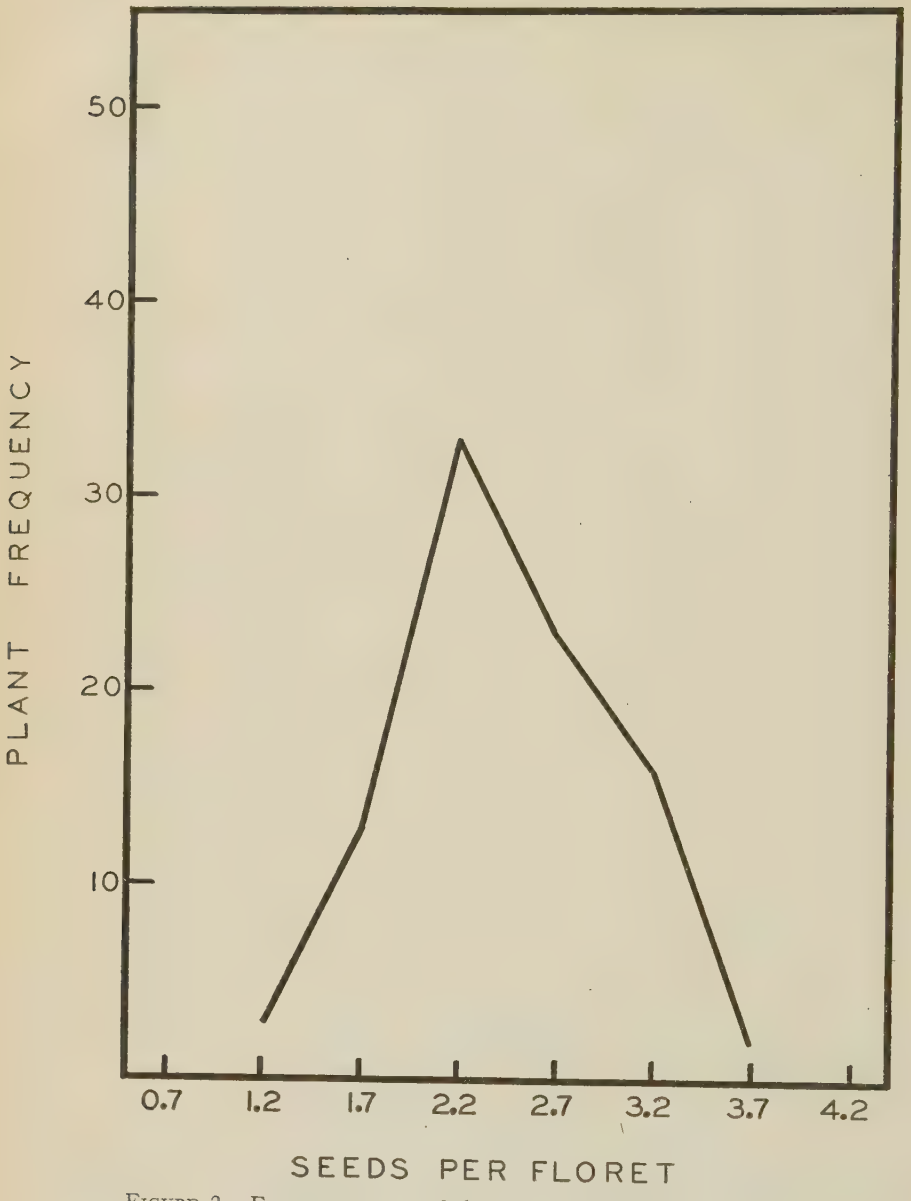

FIGURE 2. Frequency curve of the number of seeds per floret among 90 plants of pure Ladino clover in 1949.

A series of 70 clonal lines were investigated in 1949 for their seed setting ability. The results are summarized graphically in Figure 3. The mean number of seeds per floret ranged from 0.5 to 4.0, with an average of 2.3. Differences in seed setting between clons were highly significant. The frequency distribution of the number of clons in relation to the number of seeds per floret appears to be nearly normal. The clons used in this experiment, with the exception of a few, had been selected for characters other than seed setting.

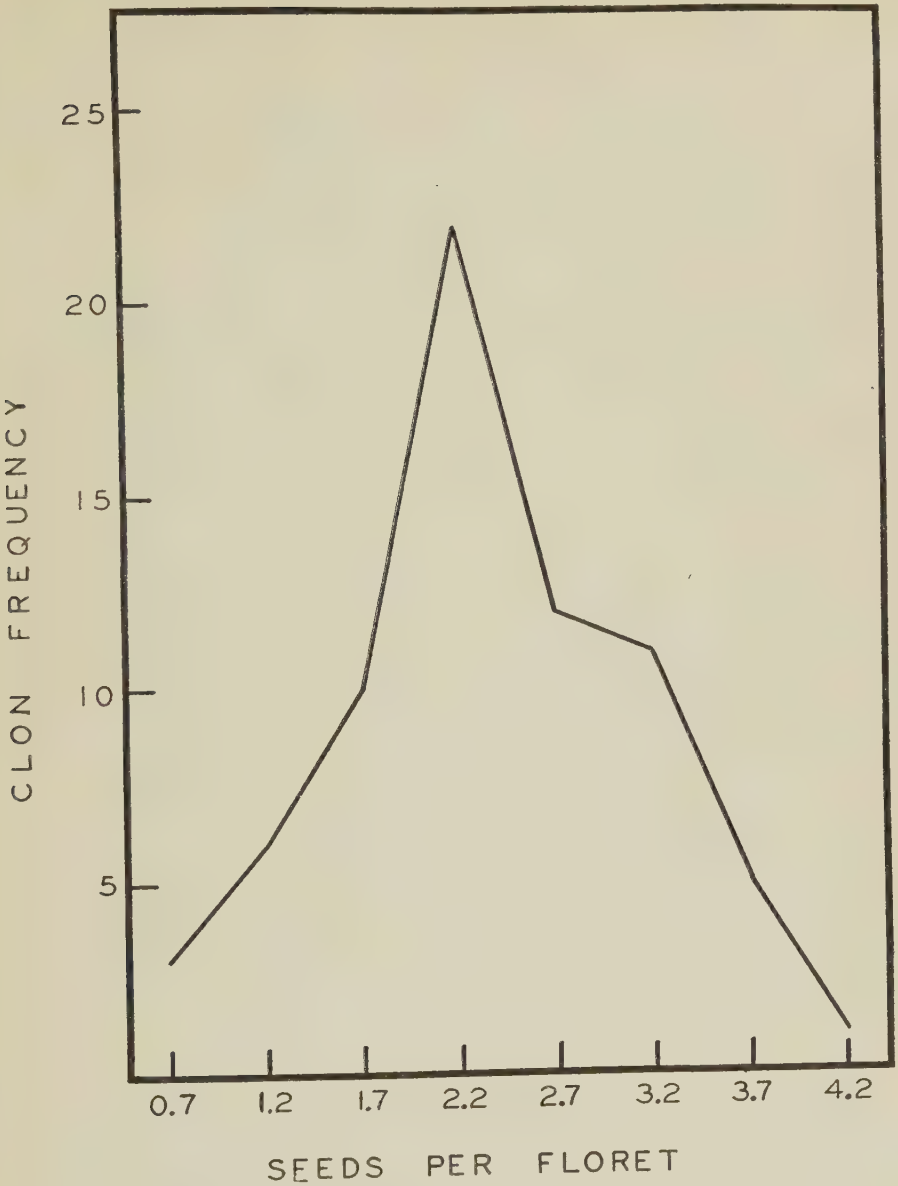

FIGURE 3. Frequency curve of the number of seeds per floret among 70 clons of Ladino clover in 1949.

Seed setting data were available in 1948 and 1949 for 40 of these clons. The 1948 data were collected from seeded single plants, whereas the 1949 data were collected from the vegetatively propagated plants. The coefficient of correlation calculated from the paired means of each clon was found to be highly significant ($r = 0.727$). High seed setting plants in 1948 remained high seed setters in 1949.

It is shown in Table 1 that an average of 89 per cent of the florets produced seeds. The percentage of seed-bearing florets was found to vary with the various plants. In 1948 it ranged from 54.5 per cent to 97.4 per cent. Among the clonal lines investigated in 1949, it ranged from 33.4 per cent to 98.3 per cent. These differences were highly significant. The wide range of variation in the percentage of seed-bearing florets indicates that all plants are not pollinated to the same extent. It is assumed that some white clover plants are more attractive to pollinating insects than others.

A comparison between the percentage of seed-bearing florets in 1948 and 1949 among the paired means of 33 clons gave a correlation coefficient of 0.530, which was highly significant. The individual plants producing a high proportion of unfertilized florets in 1948 also tended to produce a high proportion of unfertilized florets, as clon, in 1949.

The variation among plants or clons in the percentage of seed-bearing florets is reflected in the number of seeds per floret. High seed setting is usually associated with a high percentage of seed-bearing florets, whereas low seed setting is associated with a low percentage of seed-bearing florets. A low percentage of seed-bearing florets in a given plant or clon tends to decrease the number of seeds per floret. In order to eliminate this variation due to unfertilized florets, the total number of seeds for each individual was divided by the total number of seed-bearing florets. This mean number of seeds per seed-bearing floret could be taken as a seed setting rating, assuming that all florets produced seeds. The percentage of seed-bearing florets was significantly correlated with the number of seeds per seed-bearing floret. The 131 plants grown in 1948 gave a correlation of 0.620 and the 70 clons grown in 1949 a correlation coefficient of 0.657. Both were highly significant. When the percentage of seed-bearing florets is high, the number of seeds per seed-bearing floret is also high. If a high proportion of florets remains unfertilized in a given clon, the seed-bearing florets generally produce only a few seeds each, indicating that a large proportion of ovules do not develop into seeds. The degree of pollination influences not only the number of florets which set seeds but also the number of seeds set within each floret.

DISCUSSION

The problem of Ladino seed production in Eastern Canada is rather acute, due to the fact that climatic factors appear to decrease somewhat the activities of pollinating agents. It was assumed that the seed productivity of Ladino white clover could be increased by a better adaptation of the plant through breeding methods. The feasibility of this project was first determined by investigating the range of variation in seed setting among natural populations of Ladino white clover.

The influence of climatic environment on seed setting in Ladino white clover depends to a large extent on the degree of pollination. Pollination of white clover is entirely due to pollinating insects. Any climatic factors likely to change the rate of nectar or pollen-collecting activities of the pollinating agents would result into variations in seed setting.

The wide range of variation in the seed setting ability of Ladino white clover indicates that this character is highly heterozygous. The high degree of heterozygosity is maintained through the cross-pollinating habit of the crop. Variability among Ladino plants offers great opportunities for selection. Inter-crossing of the best seed setting plants should lead to the development of high seed setting strains. Differences between plants as to the degree of pollination suggest the possibility of selecting plants more completely and more effectively pollinated than others. The fact that some plants are more completely pollinated than others indicates that they may be more attractive to pollinating insects. A theoretical increase in seed production of about 50 per cent could be gained through selection for high seed setting only.

The degree of pollination is undoubtedly an important factor conditioning seed setting in white clover. It is suspected that variation between plants in regards to seed setting is not entirely due to differences in the degree of pollination. That is why other factors are now being investigated.

SUMMARY

A rather large number of individual plants and clonal lines of Ladino white clover were examined in 1948 and 1949 in order to determine the mean number of seeds per floret and the percentage of seed-bearing florets. Seed counts were made at weekly intervals with 10 Ladino plants to determine the influence of climatic environment on seed setting.

The mean number of seeds per floret and the percentage of seed-bearing florets was found to vary during the summer.

Wide differences were found between plants or clons as to the number of seeds per floret. The average number of seeds per floret appears to be around 2.4, ranging from 0.5 to 4.3. The percentage of seed-bearing florets also varies from plants to plants, and is an important factor controlling seed setting.

Selection and inter-crossing of high seed setting plants is believed to be a practical means of increasing seed production of Ladino white clover under northeastern conditions.

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THE RELATION OF FOLIAGE ANALYSIS TO KEEPING QUALITY OF McINTOSH AND SPY VARIETIES OF APPLES[1]

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With the loss of a very large proportion of the Canadian export apple market and the consequent occurrence of domestic surpluses, the production of high quality fruit has become all the more essential in order to maintain and to even increase domestic per capita consumption. Fruit quality may be affected by many factors. This paper deals with some phases of plant nutrition and their relationship to certain standards of quality in the McIntosh and Spy varieties of apple.

REVIEW OF THE LITERATURE

Probably the earliest attention paid to the possible effects of plant nutrition and directly to orchard fertilizer practice on apple quality was in the nature of observations by growers themselves. These observations, as well as those of many trained workers, have led to the widely accepted opinion that fruit from trees growing under relatively low nitrogen conditions, though reduced in numbers and small in size, is well coloured, of high quality and keeps well in cold storage. Conversely, fruit from trees growing under high nitrogen conditions, though more numerous and larger in size, is more poorly coloured, has softer flesh and has a shorter storage life.

Wallace (13) states "grass treatment of orchards generally prolongs the storage life of apples both in ordinary and low temperature storage and chemical analysis has shown that this treatment has greatly reduced the nitrogen content of the fruit". Haynes and Archibald (6) have also concluded that low nitrogen is associated with good keeping quality. Wallace (13) has further shown that under a low supply of potassium, fruits remain of dull, immature appearance, they are sub-acid, often sweet or woody and flavourless, shrivel greatly in store, and break down prematurely in low temperature stores.

Information in respect to the levels of phosphorus associated with either good tree vigour and production or quality of fruit is still not very definite. Wallace (13) states "there is still no clear evidence that phosphatic manuring produces direct response in fruit trees, though in several instances it has been shown that indirect effects may follow by stimulating the growth of leguminous cover crops". In California, Leilland (9) found that apple trees failed to respond in growth or production to applications of phosphorus on soils representing extreme cases of phosphorus deficiency with grains and leguminous grasses. Du Toit and Reynke (3) found, however, that high nitrogen applications do not interfere with keeping quality of apples if the phosphorus supply is adequate; that is if a suitable nitrogen-phosphorus ratio exists.

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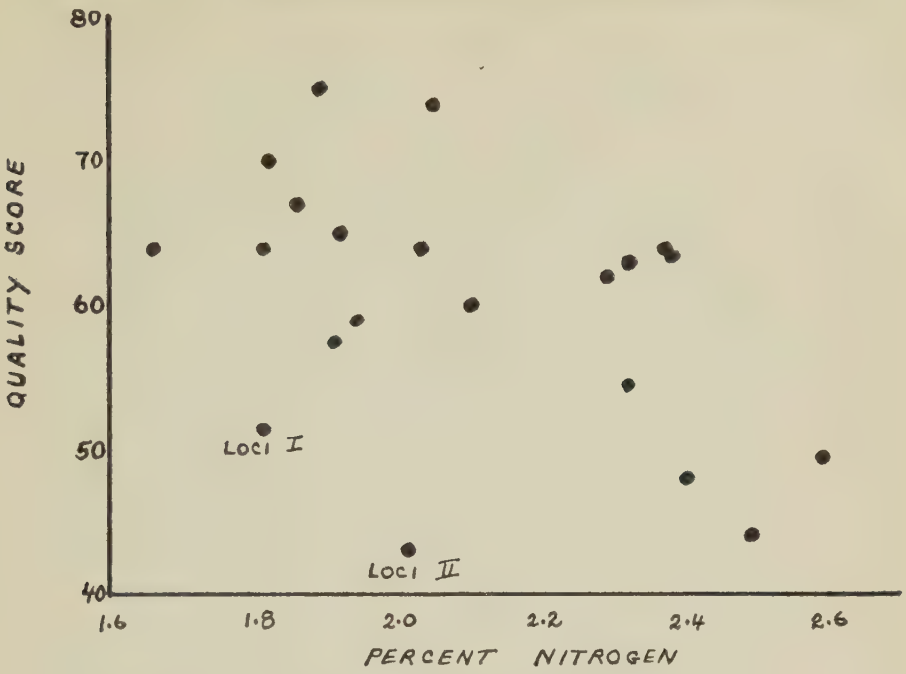

FIGURE 1. Relation between per cent foliage nitrogen and McIntosh storage quality in a group of grower orchards—1948.

Although these references indicate the possible effect of nutrition on apple quality, no definite standards were established which might be employed to advise individual growers regarding fertilizer requirements to produce quality fruit.

Later, Compton *et al.* (2) established leaf colour standards in relation to nitrogen fertilization of the McIntosh variety. A set of colour standards was published indicating the range of leaf colour associated with different levels of leaf nitrogen. It was further determined that trees whose leaves contained less than 1.8 per cent nitrogen in dry weight were low in yield and that trees whose leaves contained more than 2.1 per cent nitrogen yielded maximum crops but fruit of poor colour and generally low in quality.

With the improvement of the more rapid colorimetric methods of plant analysis it was considered that foliage analysis might serve as a basis of service fertilizer recommendations, once standards were established correlated with satisfactory yield and high fruit quality.

METHODS AND MATERIALS

The studies reported here are dealt with under two headings:

1. The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples grown in a considerable number of different orchards and under a variety of management and fertilizer use.

2. The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples from trees in a uniform orchard block and receiving specific fertilizer treatments.

Material for the first phase of the study was obtained from the following source: Bearing trees were selected from a number of orchards in a widely dispersed area in the counties of Northumberland and Durham, Ontario. Fertilizer treatment, soil and cultural conditions covered as wide a range as possible and the vigour varied from poor to excessive.

Material used in the second part of these studies was obtained from uniform trees receiving different fertilizer treatments as follows: Ammonium sulphate and superphosphate were employed at four levels in all combinations. Thus, in the data to follow, N_1P_1 represents five pounds of ammonium sulphate and two and one-half pounds of superphosphate and the higher numbers represent multiples of these basic amounts. All trees also received a basic application of one pound and a half of muriate of potash.

COLLECTION AND PREPARATION OF MATERIAL FOR ANALYSIS

Leaf tissue was collected for chemical analysis from each of the trees under test during the second or third week of July. The samples were taken at shoulder level around the circumference of the tree. Only terminal shoots were sampled and the leaves were taken from the middle third of the current year's growth. A sample of one hundred leaves was taken in this manner. The sample was oven-dried at 100°F. for twenty-four hours and was then ground to a fine powder, using at first a Waring Blendor and finally a hammer mill.

ANALYTICAL PROCEDURE

Preparation of Samples

Several methods of wet ashing were tried in order to determine nitrogen and minerals in one sample. However, it was found most satisfactory to dry-ash one sample and to make nitrogen determinations on another.

An air-dry sample equivalent to exactly two grams of dry matter was ashed in an electric muffle furnace for $2\frac{1}{2}$ hours at 550°C. The ash was digested with 4 ml. of 6N hydrochloric acid for one hour on a hot-plate, made up to 400 ml., and filtered.

Potassium Determination

The quick test method of Lindner (10), Peech and English (12) and Wolf (14) were tried but were not satisfactory. Various changes in them failed to give a satisfactory method. Finally a method which was essentially that of Garman (5) was adopted.

Garman makes use of a commercial preparation of sodium cobaltinitrite. However, in this laboratory the products of three different companies failed to give precipitates with micro-quantities of potassium. The product of a fourth company was usable but different lots varied greatly in sensitivity. After numerous trials a reagent of considerable

sensitivity and of very uniform performance was evolved. Since the details of its preparation are not immediatley available (7) they are repeated here, as follows:—

(a) 25 grams of cobalt nitrate (hydrated) were dissolved in 50 ml. of water and 16.0 ml. of glacial acetic acid added.

(b) 90 grams of sodium nitrite were dissolved in [illegible] ml. of water. To *(a)* was added all of *(b)* and the mixture was aerated at the water pump for six hours and filtered. The reagent was stable in the refrigerator for at least a month. It was aerated for a few minutes each day before use. Best results were obtained when the potassium did not exceed 0.8 mg. Results were within an average range of ±4 per cent when compared with quantitative results obtained by the method of McCance and Shipp (11).

Magnesium Determination

The titan yellow method of Peech and English (1) was used with results agreeing with about ±10 per cent of determinations by quantitative methods.

Phosphorus Determination

Silica is not removed in the preparation of the samples. Consequently the method of King (8) was adopted and found very satisfactory.

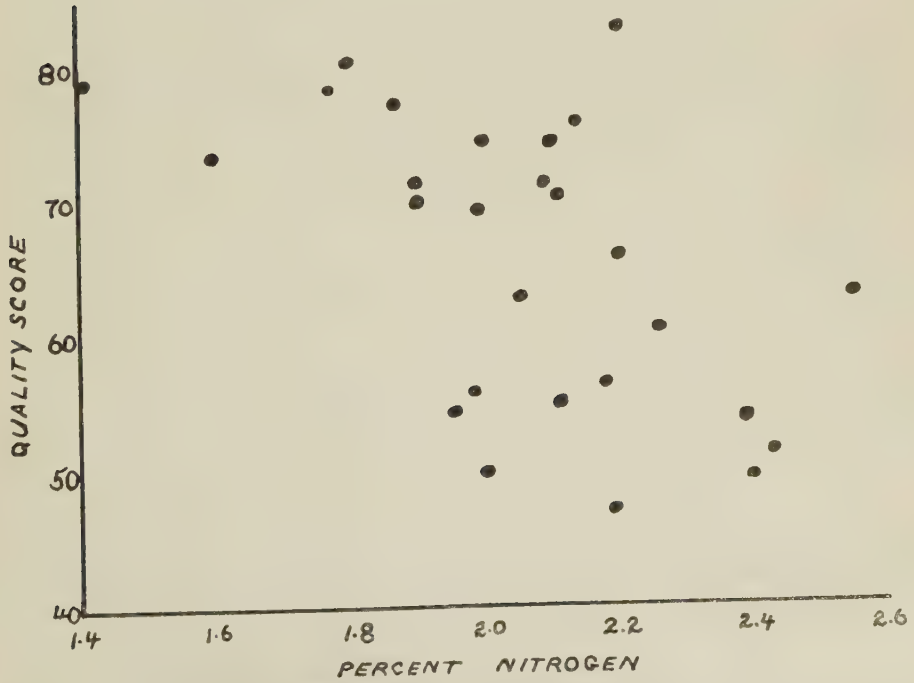

FIGURE 2. Relation between per cent foliage nitrogen and McIntosh storage quality in a group of grower orchards—1949.

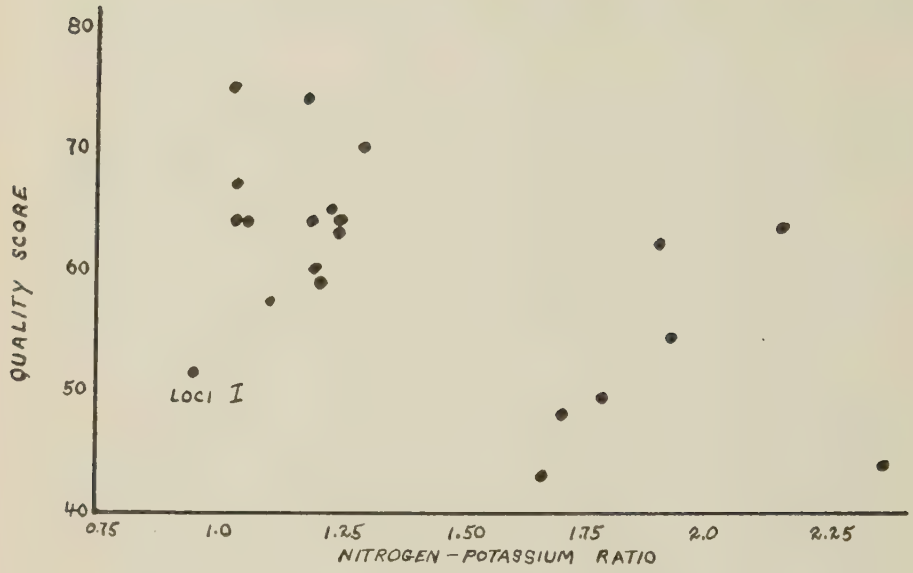

FIGURE 3. Relation between the nitrogen-potassium ratio and McIntosh storage quality in a group of grower orchards—1948.

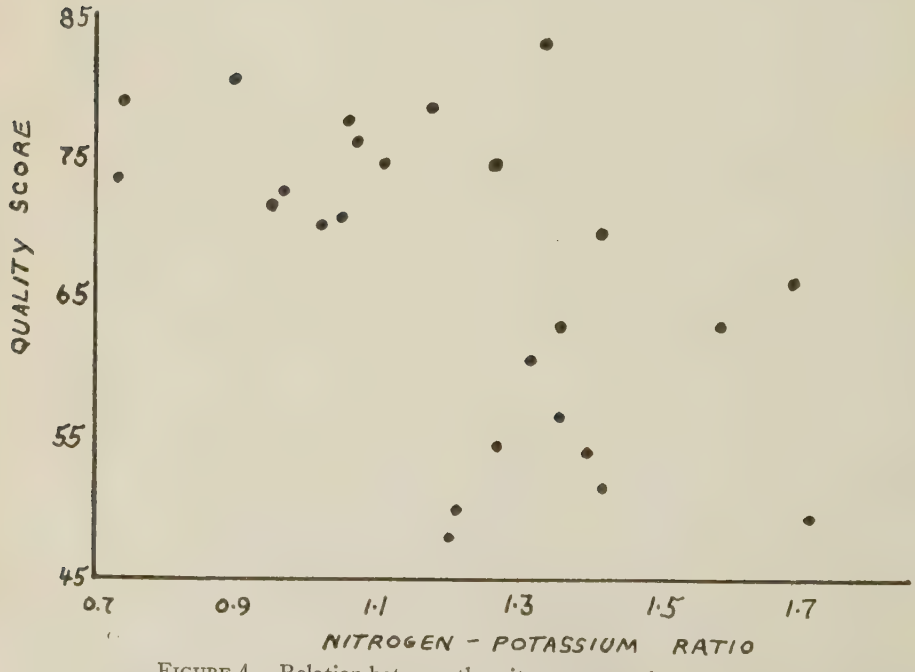

FIGURE 4. Relation between the nitrogen-potassium ratio and McIntosh storage quality in a group of grower orchards—1949.

Nitrogen Determination

Nitrogen was determined by the ordinary Kjeldahl procedure or by a nesslerization method which has proven very rapid and has given results in good agreement with the Kjeldahl method.

An 0.5 gm. sample was placed in a 125 ml. erlenmeyer flask with 10 ml. of the digestion mixture of Campbell and Hanna (1). Glass beads were added, the flask heated gently until foaming occurred, and the flame withdrawn until foaming ceased. Boiling was continued until the solution became a clear, pale green, then about two minutes longer—usually a total time of ten to twelve minutes. The digest was diluted after cooling to 100 ml., and a 10 ml. aliquot further diluted to 100 ml. A 1 ml. aliquot was measured into a colorimeter tube, 0.5 ml. of Rochelle salt solution (50 grams in 100 ml. of water) added, and the volume made up to 6 ml. After mixing, 1 ml. of Nessler's reagent—the reagent of Folin and Wu (4) diluted 1 to 1 with water—was added, the whole mixed and an immediate reading made with a blue filter. Comparison with the Kjeldahl method gave results with ± 4 per cent, and an average deviation of ± 2 per cent.

Collection of Fruit for Storage

Fruit was collected around the circumference of the tree in the same manner as the leaf tissue. This fruit was collected as close as possible to the date at which the grower picked the rest of the orchard. Maturity readings as indicated by the iodine starch test were made at the time of picking. The fruit was placed in cold storage at 32°F., generally within 48 hours after picking.

Methods Used in Evaluating Keeping Quality of Fruit

While the results herein reported are confined to studies conducted in 1948 and 1949, similar studies were also carried out in 1946 and 1947. During the first year of the test the storage quality was based in the case

TABLE 1.—QUALITY EVALUATIONS

Date.....Sample.....

		Values	*Max. Score*
(1) Flavour (aroma-acidity)	0 – 10	very poor	30
	10 – 20	poor–medium	
	20 – 30	medium–very good	
(2) Texture (juiciness–mealiness)	0 – 10	very mealy	30
	10 – 20	mealy	
	20 – 30	mellow crisp	
(3) Appearance			
blush	0 – 10	very poor–poor	30
colour quality	10 – 20	poor–medium	
blemish	20 – 30	medium–very good	
(4) Miscellaneous			
hardness			3
greasiness			3
gr. colour		yellow = max. score	3

TABLE 2.—KEEPING QUALITY OF MCINTOSH APPLES GROWN UNDER A VARIETY OF CONDITIONS IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Orchard No.	1948						1949					
	Quality	Spoilage	N%	P%	K%	Mg%	Quality	Spoilage	N%	P%	K%	Mg%
1	—	—	—	—	—	—	83.0	28	2.22	0.19	1.65	0.17
2	64	21	2.03	0.19	1.70	0.17	80.5	70	1.80	0.18	2.00	0.18
3	—	—	—	—	—	—	79.0	3	1.41	0.17	1.90	0.22
4	64	0	1.81	0.17	1.70	0.24	78.5	78	1.77	0.17	1.50	0.23
5	75	7	1.89	0.21	1.70	0.24	77.5	65	1.87	0.19	1.75	0.34
6	64	86	1.66	0.34	1.60	0.26	76.0	120	2.14	0.34	2.00	0.18
7	54.5	74	2.32	0.18	1.20	0.28	74.5	21	2.00	0.20	1.80	0.32
8	63.5	8	2.38	0.17	1.10	0.27	74.5	70	2.10	0.19	1.65	0.30
9	67	23	1.86	0.33	1.65	0.34	73.5	26	1.60	0.35	2.20	0.22
10	74	26	2.13	0.26	1.80	0.25	72.5	119	2.09	0.22	2.15	0.28
11	—	—	—	—	—	—	71.5	20	1.90	0.19	2.00	0.27
12	—	—	—	—	—	—	70.5	98	2.11	0.23	2.00	0.32
13	63	23	2.32	0.16	1.85	0.18	70.0	20	1.90	0.22	1.85	0.20
14	—	—	—	—	—	—	69.5	153	1.99	0.26	1.40	0.26
15	62	15	2.29	0.19	1.20	0.28	66.0	110	2.20	0.19	1.30	0.35
16	64	0	2.37	0.22	1.90	0.23	63.0	64	2.55	0.23	1.60	0.34
17	49.5	27	2.59	0.21	1.45	0.24	63.0	5	2.05	0.18	1.50	0.30
18	—	—	—	—	—	—	60.5	97	2.26	0.17	1.70	0.20
19	65	57	1.92	0.14	1.55	0.26	56.5	16	2.18	0.17	1.60	0.31
20	43	58	2.01	0.16	1.20	0.23	56.0	2	1.98	0.27	1.20	0.27
21	—	—	—	—	—	—	55.0	47	2.11	0.21	1.90	0.23
22	—	—	—	—	—	—	54.5	124	1.95	0.20	1.45	0.27
23	—	—	—	—	—	—	54.0	117	2.39	0.19	1.70	0.30
24	48	74	2.40	0.18	1.40	0.30	51.5	133	2.43	0.25	1.70	0.30
25	59	87	1.94	0.19	1.60	0.23	50.0	160	2.00	0.24	1.65	0.30
26	44	0	2.49	0.18	1.05	0.30	49.5	27	2.40	0.22	1.40	0.30
27	60	20	2.10	0.13	1.75	0.23	47.0	117	2.09	0.20	1.75	0.27
28	70	23	1.82	0.17	1.40	0.24						
29	57.5	12	1.91	0.15	1.70	0.20						
30	51.5	124	1.81	0.31	1.90	0.21						

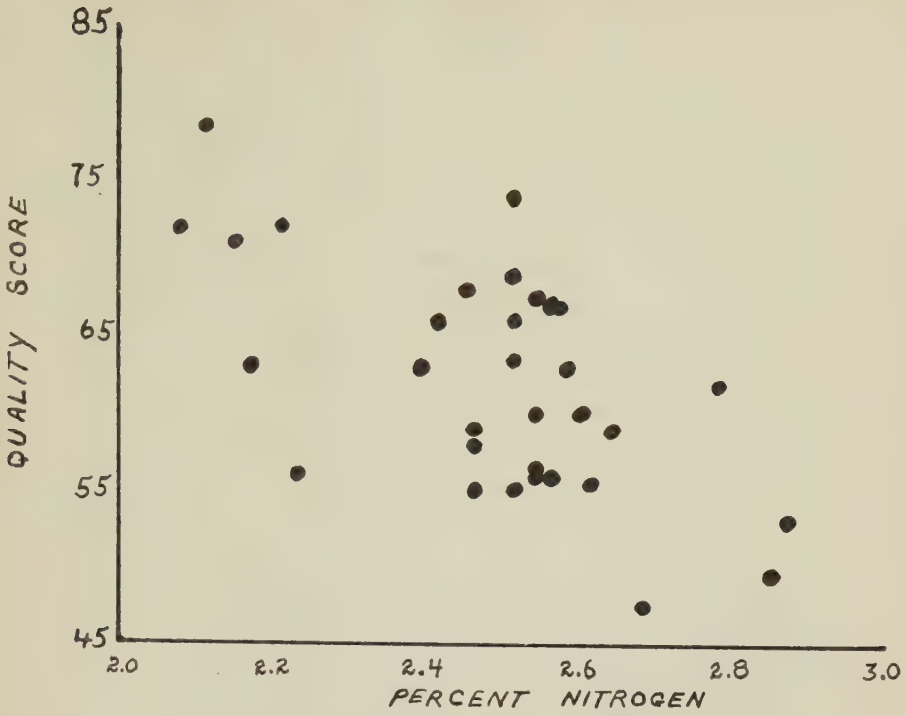

FIGURE 5. Relation between per cent foliage nitrogen and McIntosh storage quality in a uniform orchard block—1948.

of the McIntosh variety on the core flush index, occurring in April after the fruit had been removed from cold storage and placed at 70°F. for one week. While the appearance of core flush may be accepted as an expression of approaching physiological senility it is only after an extended period in storage that samples may be evaluated by this index. In respect to providing the market with apples of acceptable quality the variety should be marketed long before this period. It was therefore considered that other indexes of quality such as general appearance, texture of flesh, flavour, etc., might have more influence on the estimation of quality by the buying public. With this in mind the quality score in Table I was adopted, while the occurrence of core flush in McIntosh and bitter-pit in Spy was retained as the main constituent of the spoilage index.

RESULTS

I. The relation between foliage analysis and the keeping quality of McIntosh and Spy apples grown under a variety of conditions.

The results of foliage analysis, storage quality and spoilage index of the McIntosh variety for 1948 and 1949 are given in Table 2. In both years there were definite negative correlations between the nitrogen content of the foliage and storage quality of the fruit (Figures 1 and 2). In 1948 the correlation coefficient between nitrogen content and fruit quality was $r = -.6153^{**}$ and in 1949, $r = -.374^{*}$. Referring to Figure 1, the position

** Two asterisks following a coefficient of correlation indicate odds greater than 99 : 1 (highly significant).
* One asterisk represents odds between 99 : 1 and 19 : 1 (significant).

TABLE 3.—KEEPING QUALITY OF SPY APPLES GROWN UNDER A VARIETY OF CONDITIONS IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Orchard No.	1948						1949					
	Quality Score	Spoilage Index	N%	P%	K%	Mg%	Quality Score	Spoilage Index	N%	P%	K%	Mg%
1	45.5	0	2.40	0.16	2.05	0.19	85	17	2.1	0.21	2.15	0.20
2	—	—	—	—	—	—	84.5	28	—	—	—	—
3	71	4	2.16	0.18	1.25	0.23	75.0	0	2.3	0.21	1.55	0.28
4	—	—	—	—	—	—	74.0	12	—	—	—	—
5	—	—	—	—	—	—	72.5	0	—	—	—	—
6	62	161	1.79	0.23	1.85	0.22	72.5	43	2.32	0.26	2.60	0.23
7	65	3	2.43	0.20	1.80	0.22	72.5	33	2.40	0.21	2.00	0.25
8	76	21	2.29	0.25	1.80	0.33	72.0	68	2.01	0.23	1.80	0.21
9	65	11	2.23	0.35	1.95	0.28	70.0	8	2.10	0.17	1.60	0.28
10	78	20	2.69	0.22	1.75	0.30	67.0	4	2.20	0.18	1.80	0.37
11	—	—	—	—	—	—	62.0	5	2.30	0.23	2.15	0.27
12	81.5	67	1.91	0.34	1.95	0.22	58.5	13	2.64	0.53	2.10	0.19
13	65.5	16	2.23	0.30	1.70	0.28	—	—	—	—	—	—
14	68.0	14	2.23	0.31	1.75	0.26	48.5	2	2.58	0.25	1.80	0.25
15	59.5	46	2.23	0.34	2.00	0.17	—	—	—	—	—	—

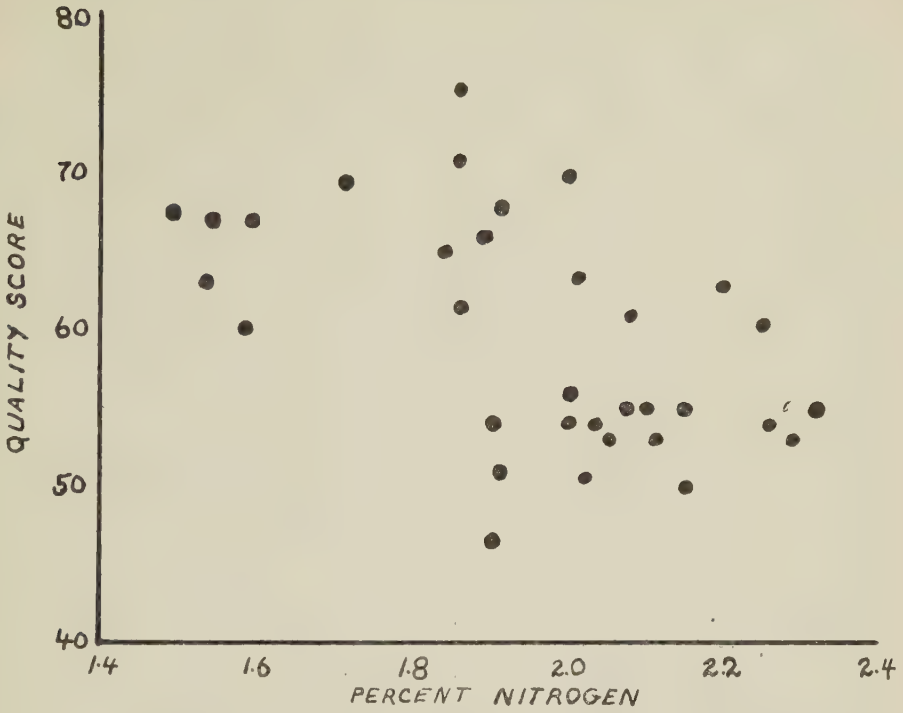

FIGURE 6. Relation between per cent foliage nitrogen and McIntosh storage quality in a uniform orchard block—1949.

in the graph of locus one and two may be accounted for by factors other than the nitrogen content of the foliage. Locus one represents a very closely planted orchard which had been lightly pruned so that quality, especially colour, was low due to poor penetration of light. Locus two represents an orchard which had a particularly low level of potassium in the foliage and it is considered that the supply of this element was the main factor in the quality score.

In 1948 it is apparent that when nitrogen alone is considered, the quality of the fruit is reduced if the nitrogen level in the foliage is greater than 2.0 to 2.1 per cent. The 1949 results largely confirm this relationship.

If the nitrogen values are divided into two categories, below and above 2.1 per cent, there is no correlation in 1948 with nitrogen below this point and a negative correlation with quality above this level of nitrogen in both years (1948 $r = -.8124^{**}$; 1949 $r = -.4419^{*}$).

The spoilage index which is derived from the amount and intensity of core flush did not show any constant relationship either with the levels of nitrogen in the foliage or with the quality score of the stored fruit. This fact is apparent on examination of the data for the two years.

In both 1948 and 1949 there was a positive trend between potassium levels in the foliage up to 1.7 per cent of dry matter and increased quality of the fruit. Whereas the single effects of nitrogen and potassium both exert an effect, such single effects are somewhat modified by the ratio of nitrogen to potassium (Figures 3 and 4). In both years fruit from trees

TABLE 4.—EFFECT OF FERTILIZER APPLICATIONS ON FRUIT QUALITY AND FOLIAGE NUTRIENT LEVELS IN 1948 AND 1949 EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	McIntosh—1948						McIntosh—1949					
	N%	P%	K%	Mg%	N/K Ratio	Quality Score	N%	P%	K%	Mg%	N/K Ratio	Quality Score
N_1	2.21	0.25	1.82	0.22	1.21	64.4	1.67	0.23	1.68	0.21	0.99	64.8
N_2	2.53	0.21	1.57	0.24	1.61	53.2	1.98	0.18	1.51	0.25	1.29	59.5
N_3	2.62	0.21	1.44	0.24	1.82	53.0	2.03	0.17	1.45	0.26	1.39	58.0
N_4	2.54	0.20	1.43	0.24	1.78	55.4	2.12	0.17	1.42	0.28	1.49	56.9
P_1	2.49	0.21	1.60	0.22	1.59	58.7	1.99	0.19	1.58	0.23	1.27	55.3
P_2	2.45	0.21	1.49	0.24	1.67	53.6	1.96	0.18	1.51	0.26	1.30	60.6
P_3	2.54	0.22	1.58	0.23	1.63	57.9	1.97	0.18	1.48	0.25	1.34	61.2
P_4	2.45	0.22	1.56	0.25	1.58	53.4	1.84	0.20	1.48	0.26	1.25	62.5

Treatment	Spy—1948						Spy—1949					
	N%	P%	K%	Mg%	N/K Ratio	Quality Score	N%	P%	K%	Mg%	N/K Ratio	Quality Score
N_1	2.24	0.29	1.86	0.22	1.20		1.85	0.30	1.80	0.22	1.03	76.8
N_2	2.55	0.23	1.60	0.23	1.59		1.98	0.23	1.67	0.23	1.18	71.2
N_3	2.71	0.22	1.63	0.23	1.66		2.18	0.20	1.66	0.24	1.32	60.1
N_4	2.81	0.21	1.51	0.24	1.86	No	2.18	0.19	1.57	0.22	1.38	62.1
P_1	2.46	0.25	1.66	0.22	1.48	Crop	2.00	0.25	1.70	0.22	1.19	67.4
P_2	2.66	0.22	1.62	0.24	1.64		2.02	0.21	1.64	0.25	1.24	68.3
P_3	2.64	0.24	1.67	0.22	1.52		2.06	0.22	1.65	0.23	1.23	69.7
P_4	2.62	0.25	1.66	0.23	1.58		2.12	0.24	1.66	0.23	1.28	65.0

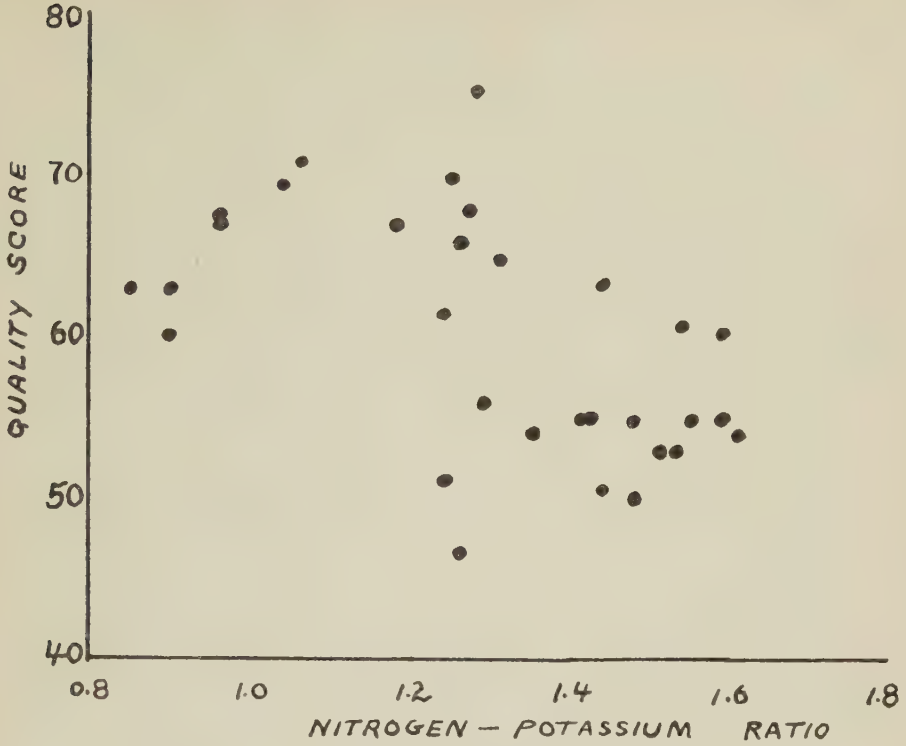

FIGURE 7. Relation between nitrogen-potassium ratio and McIntosh storage quality in a uniform orchard block—1949.

whose foliage had a nitrogen-potassium ration of not more than 1.25 had a significantly higher quality score than those where the nitrogen-potassium ratio was above this figure.

Nitrogen above the 2.1 per cent level would have much more effect in reducing fruit quality if the potassium content was also low and even this level of nitrogen might prove excessive under such conditions.

No relationship was found between the phosphorus content of the foliage and the quality score or spoilage index in either 1948 or 1949.

Results with the Spy Variety

The results of the foliage analysis, storage quality and spoilage index of the Spy variety are recorded in Table 3. No definite relationships were established between any of the elements determined in the foliage and fruit quality, although in 1949 the two samples having the lowest quality score had the highest nitrogen content in the foliage.

II. The relation between foliage analysis and the keeping quality of McIntosh and Spy varieties of apples from trees in a uniform orchard block receiving specific fertilizer treatments.

In addition to the direct relationships between foliage analysis of individual trees and storage quality of the fruit the effect of fertilizer applications on the foliage constituents and fruit quality is of interest and the pertaining data are recorded in Table 4.

TABLE 5.—KEEPING QUALITY OF MCINTOSH APPLES FROM A UNIFORM ORCHARD BLOCK IN 1948 AND 1949 IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	1948						1949					
	Quality Score	Spoilage Index	N%	P%	K%	Mg%	Quality Score	Spoilage Index	N%	P%	K%	Mg%
N_1P_1	67	230	2.21	0.25	1.75	0.21	60	155	1.58	0.23	1.75	0.20
	69	81	2.51	0.23	2.10	0.16	53	216	2.05	0.21	2.00	0.16
N_1P_2	64	84	—	—	—	—	71	135	1.86	0.21	1.75	0.18
	67	92	2.08	0.24	1.90	0.23	63	121	1.53	0.25	1.80	0.25
N_1P_3	66	95	2.15	0.26	1.85	0.22	69.5	126	1.71	0.24	1.65	0.24
	73.5	34	2.11	0.23	1.75	0.22	67.5	99	1.48	0.22	1.55	0.19
N_1P_4	51	110	2.23	0.24	1.80	0.27	67	116	1.59	0.23	1.65	0.26
	58	66	2.17	0.30	1.65	0.26	67	78	1.54	0.26	1.30	0.26
N_2P_1	63	113	2.46	0.21	1.65	0.18	46.5	108	1.90	0.16	1.50	0.19
	54	117	2.64	0.20	1.65	0.22	70	128	2.00	0.17	1.60	0.22
N_2P_2	63	136	2.45	0.22	1.50	0.26	66	111	1.89	0.17	1.40	0.27
	33	205	2.54	0.22	1.50	0.25	61.5	118	1.86	0.17	1.50	0.28
N_2P_3	61	132	2.51	0.21	1.60	0.22	55	154	2.32	0.19	1.65	0.29
	55	186	2.54	0.22	1.50	0.24	55	88	2.10	0.19	1.35	0.22
N_2P_4	54	51	2.43	0.25	1.60	0.27	68	131	1.91	0.19	1.50	0.30
	42.5	138	2.68	0.20	1.60	0.28	54	76	1.90	0.21	1.65	0.29
N_3P_1	50.5	138	2.61	0.22	1.40	0.28	—	—	2.20	0.18	1.50	0.25
	50	126	2.51	0.22	1.48	0.21	50.6	53	2.02	0.18	1.40	0.31
N_3P_2	51	49	2.54	0.20	1.50	0.23	50.0	75	2.15	0.16	1.45	0.25
	51	76	2.56	0.21	1.30	0.26	55.0	117	2.15	0.17	1.45	0.28
N_3P_3	57	184	2.78	0.21	1.65	0.22	56	133	2.00	0.18	1.55	0.23
	44.5	90	2.85	0.20	1.40	0.25	65	136	1.84	0.15	1.40	0.26
N_3P_4	58	184	2.58	0.22	1.40	0.25	75.5	107	1.86	0.19	1.45	0.25
	62.5	77	2.54	0.21	1.40	0.25	54.0	189	2.00	0.19	1.40	0.26
N_4P_1	55	53	2.60	0.20	1.42	0.23	54.0	143	2.03	0.19	1.50	0.31
	61	112	2.41	0.21	1.35	0.27	53.0	127	2.11	0.18	1.40	0.22
N_4P_2	62	126	2.57	0.22	1.50	0.27	63.0	98	2.20	0.17	1.50	0.27
	58	47	2.39	0.20	1.25	0.26	55.0	84	2.07	0.16	1.30	0.30
N_4P_3	48	163	2.87	0.21	1.48	0.25	60.5	119	2.23	0.16	1.40	0.30
	58.5	78	2.51	0.20	1.40	0.24	61.0	85	2.08	0.15	1.35	0.29
N_4P_4	50	162	2.46	0.19	1.60	0.22	51.0	136	1.91	0.16	1.50	0.30
	51	88	2.54	0.20	1.45	0.24	63.5	129	2.01	0.15	1.40	0.30

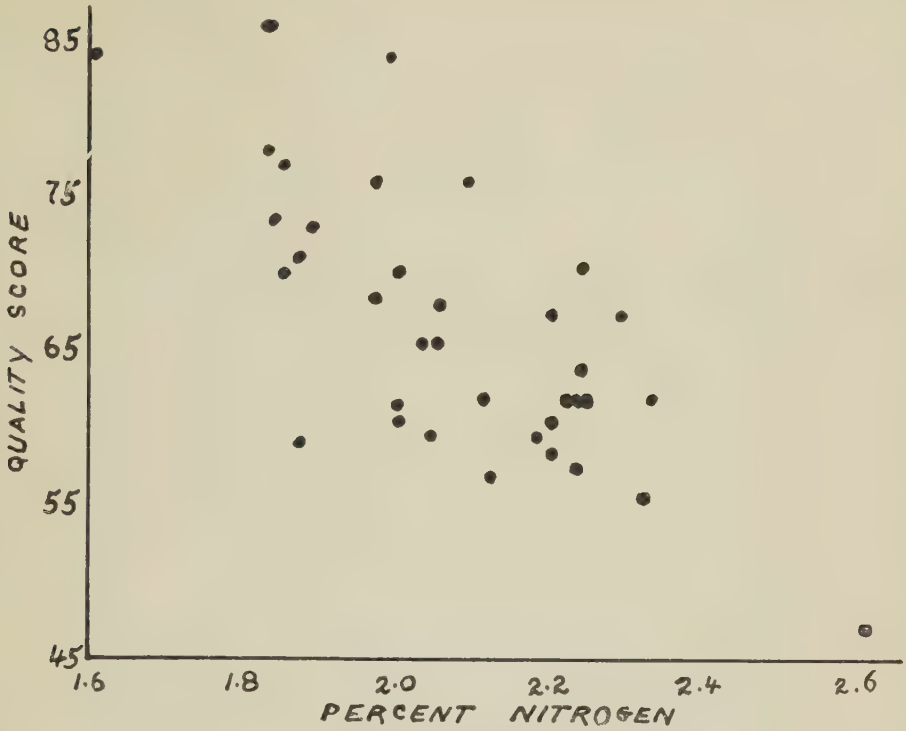

FIGURE 8. Relation between per cent foliage nitrogen and Spy storage quality in a uniform orchard block—1949.

It will be noted that in both varieties progressive increments of ammonium sulphate have brought about a steady increase of nitrogen in the foliage. This increase of nitrogen content is accompanied by a considerable decrease of potassium and phosphorus and increase of magnesium. This increase in nitrogen content and the widening of the N/K ratio is associated with a decrease in the fruit quality score. In the case of McIntosh the first additional increment of ammonium sulphate (10 lb. per tree) has brought about the largest decrease in quality, while in the Spy variety the largest decrease does not take place in 1949 until an application of fifteen pounds per tree, although the N/K ratio is approximately the same in both cases. The significance of maintaining an adequate potassium supply with high nitrogen feeding should be emphasized. Progressive increments of superphosphate had no consistent effect upon the phosphorus, nitrogen or potassium content of the foliage or quality score of the fruit.

The results of the foliage analysis, quality score and spoilage index for individual trees of the McIntosh variety are recorded in Table 5. In both years there were definite negative correlations between the nitrogen content of the foliage and storage quality of the fruit. (Figures 5 and 6). In 1948 the correlation coefficient between nitrogen content and fruit quality was $r = -.5762^{**}$ and in 1949 $r = -.5173^{**}$. In an examination of Figures 5 and 6 it will be apparent that the quality of the fruit was reduced if the nitrogen level in the foliage was greater than 2.0 to 2.1 per cent. This result is similar to that obtained in the first phase of this study.

TABLE 6.—KEEPING QUALITY OF SPY APPLES FROM A UNIFORM ORCHARD BLOCK IN 1949 IN RELATION TO FOLIAGE ANALYSIS EXPRESSED AS PERCENTAGE OF DRY MATTER

Treatment	Quality Score	Bitter Pit Index	N%	P%	K%	Mg%
N_1P_1	84.0	14	1.61	0.34	2.10	0.25
	68.5	9	1.97	0.29	2.00	0.22
N_1P_2	70.0	19	1.85	0.29	1.55	0.23
	73.0	6	1.89	0.25	1.75	0.27
N_1P_3	78.0	18	1.83	0.36	1.85	0.17
	84.0	26	1.99	0.21	1.55	0.20
N_1P_4	86.0	23	1.83	0.34	1.80	0.21
	71.0	3	1.87	0.33	1.80	0.23
N_2P_1	77.0	1	1.85	0.34	1.70	0.24
	59.0	0	1.87	0.23	1.60	0.20
N_2P_2	76.0	3	1.97	0.21	1.65	0.27
	—	—	—	—	—	—
N_2P_3	70.0	7	2.00	0.21	1.80	0.21
	76.0	6	2.09	0.20	1.70	0.24
N_2P_4	73.5	17	1.84	0.22	1.65	0.23
	67.5	25	2.29	0.20	1.60	0.25
N_3P_1	62.0	23	2.23	0.21	1.55	0.23
	59.5	3	2.04	0.20	1.65	0.23
N_3P_2	61.5	17	2.00	0.19	1.80	0.23
	70.5	35	2.24	0.20	1.40	0.25
N_3P_3	65.5	4	2.05	0.20	1.60	0.24
	58.5	8	2.20	0.19	1.80	0.27
N_3P_4	57.0	1	2.12	0.21	1.80	0.24
	47.0	5	2.61	0.20	1.70	0.27
N_4P_1	62.0	5	2.23	0.20	1.55	0.22
	67.5	10	2.20	0.20	1.50	0.19
N_4P_2	68.0	1	2.05	0.18	1.60	0.24
	59.5	0	2.18	0.21	1.75	0.26
N_4P_3	62.0	4	2.11	0.20	1.70	0.25
	64.0	6	2.24	0.20	1.45	0.25
N_4P_4	60.5	0	2.20	0.21	1.65	0.20

In the 1948 results there is also a progressive increase in quality with levels of potassium up to 1.7 per cent of dry matter. As in the first phase of this study single effects of nitrogen and potassium were somewhat modified by the ratio of nitrogen to potassium. An examination of Figure 7 shows that as the ratio of nitrogen to potassium increased beyond 1.25 to 1.30, a decrease in fruit quality occurred.

There was no apparent relationship between the phosphorus content of the foliage and storage quality of the fruit.

Results with the Spy Variety

The results of foliage analysis, fruit quality and spoilage index are recorded in Table 6. There is a very highly significant correlation ($r = -.9439^{**}$) between the nitrogen level in the foliage and the quality score of the fruit. There does not appear to be a sharp break in this relationship, although the marked decrease in quality occurs with nitrogen levels above 2.0 to 2.1 per cent, as shown in Figure 8. Although there was no direct relationship apparent between the potassium level in the foliage and fruit quality there was a significant negative correlation ($r = -.5703^{**}$) between the nitrogen-potassium ratio and quality as shown in Figure 9. There is a considerable decrease in quality with a nitrogen-potassium ratio greater than 1.2.

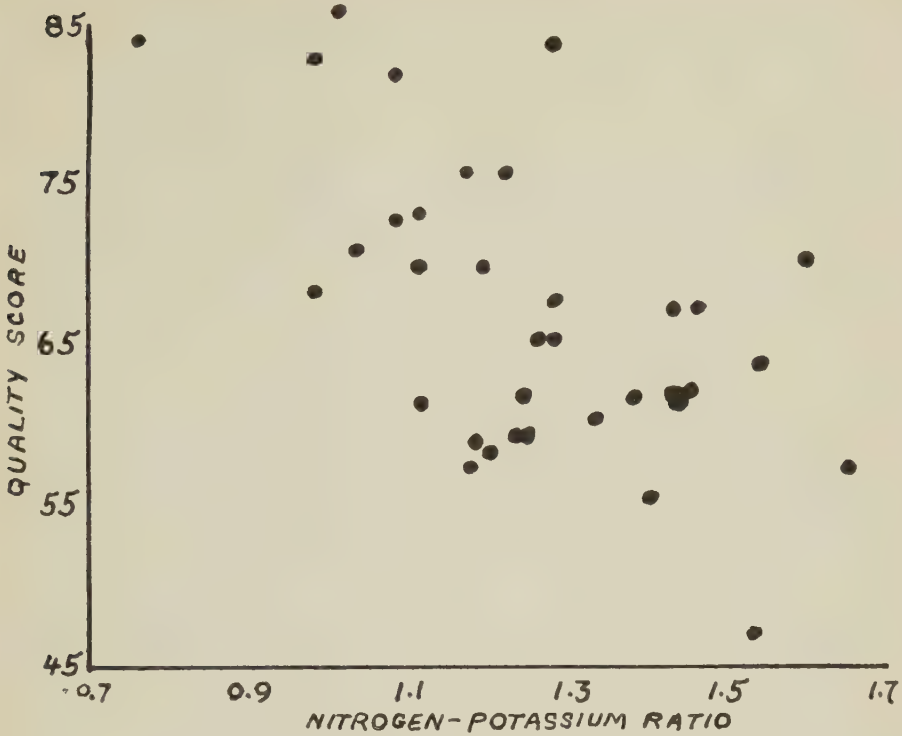

FIGURE 9. Relation between nitrogen-potassium ratio and Spy storage quality in a uniform orchard block—1949.

SUMMARY AND CONCLUSIONS

The relation between foliage analyses for nitrogen, potassium, phosphorus, magnesium and keeping quality of McIntosh and Spy apples was studied with material derived from two main sources: from individual trees located in a considerable number of different orchards under a variety of management and fertilizer use and secondly, from trees in a uniform orchard block receiving specific fertilizer treatments.

In the first phase of the study with the McIntosh variety a significant negative correlation was obtained between the nitrogen content of the foliage and the quality score of the fruit. When only nitrogen was considered the quality of the fruit was reduced if the nitrogen level in the foliage exceeded 2.0 to 2.1 per cent. There was a positive trend between potassium levels in the foliage up to 1.7 per cent of dry matter and fruit quality. Fruit from trees whose foliage had a nitrogen-potassium ratio of not more than 1.25 had a significantly higher quality score than samples where the nitrogen-potassium ratio was above this figure.

No relationship was established between the phosphorus content of the foliage and quality score or spoilage index of the fruit.

In the Spy variety no definite relationships were established between foliage analyses and fruit quality.

In the second phase of the study the application of increasing increments of nitrogen brought about a steady increase of nitrogen in the foliage, accompanied by a considerable decrease of potassium and phos-

phorus. The widening of the N/K ratio was associated with a decrease in fruit quality. Progressive increments of superphosphate had no consistent effect upon the phosphorus, nitrogen or potassium content of the foliage.

In both the McIntosh and Spy varieties there was a definite negative correlation between the level of nitrogen in the foliage and the quality score of the fruit. As in the first phase of the study the critical level appeared to be approximately 2.0 to 2.1 per cent. There was also a decrease in the fruit quality if the nitrogen-potassium ratio exceeded 1.25.

There was no apparent relationship between the phosphorus content of the foliage and the storage quality of the fruit.

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